

Please cite the Published Version

Almashali, Malak Ataaalah (2018) Expediting the confirmation of acute myocardial infarction with point of care troponin and heart fatty acid binding protein testing to facilitate early intervention in emergency department. Doctoral thesis (PhD), Manchester Metropolitan University.

Downloaded from: <https://e-space.mmu.ac.uk/621967/>

Usage rights:



[Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0](#)

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

**Expediting the confirmation of acute myocardial infarction with point of
care troponin and heart fatty acid binding protein testing to facilitate
early intervention in emergency department**

A thesis submitted in partial fulfilment of the requirements of the Manchester
Metropolitan University for the degree of
Doctor of Philosophy

Malak Ataaalah S Almashali

School of Healthcare Science

Manchester Metropolitan University

April 2018

Table of contents

Table of contents	i
List of figures.....	vii
List of tables.....	ix
Abstract.....	xiii
Declaration.....	xv
List of publications and achievements derived from this project	xvi
Acknowledgements.....	xvii
List of Abbreviations.....	xix
Chapter 1 : Introduction	1
1.1. Background.....	2
1.2. The pathophysiology of coronary heart disease	5
1.3. Myocardial infarction	9
1.3.1. ECG: AMI classification.....	11
1.4. Acute coronary syndrome assessment and management	14
1.4.1. Risk factors for acute coronary syndrome	14
1.4.2. The use of risk scores for stratification of acute coronary syndrome patients	15
1.4.3. Decision aids derived in patients with ACS	16
1.5. Risk Stratification and Clinical Decision-making	25
1.6. Cardiac Biomarkers: Historical Perspective	27
1.7. AMI Definitions: Historical Perspective	29
1.8. Cardiac Markers: Characteristics.....	30
1.9. Heart-type fatty acid-binding protein	31
1.10. Ruling out ACS: Combining Troponin and h-FABP	32
1.11. Cardiac Troponin	35
1.12. Myocardial Injury: Time of Protein Release	39

1.13. Cardiac Troponin Assays.....	40
1.14. Point-of-care testing	42
1.15. Accelerated chest pain protocols.....	46
Aim and objectives.....	47
Chapter 2 : Materials and Methods.....	50
2.1. Methods.....	51
2.1.1. Detailed plan of investigation	51
2.1.2. Patient consent.....	53
2.1.3. Data collection.....	53
2.1.4. Procedures for blood sampling and data recording.....	54
2.1.5. Blood sample labelling and storage.....	57
2.1.6. Data recording.....	57
2.1.7. Ethical approval and Considerations.....	57
2.1.8. Follow up after 30 days	58
2.1.9. Tracking of missing data	58
2.1.10. Outcome measures.....	59
2.1.12. Sample size/Power Calculations.....	60
2.1.11. Statistical techniques.....	60
2.2. Materials	61
2.2.1. Equipment and consumables	61
2.2.2: i-STAT - Instrument Description and System Overview	61
2.2.3 The FABPulous B.V. (Maastricht, Netherlands) point of care device for Heart Fatty Acid Binding Protein detection	64
2.2.4 High sensitivity troponin assay and contemporary troponin assays	68
Chapter 3 : The inter-observer reliability of a novel qualitative point of care assay for heart-type fatty acid binding protein	69
Abstract	70

3.1. Introduction.....	71
3.1.1. Aim and objectives	75
3.2. Method.....	75
3.2.1. Data collection and processing	75
3.2.3. Statistical methods	76
3.2.4 Sample size calculation	77
3.3. Results.....	78
3.3.1. Baseline characteristics.....	78
3.3.2. Result interpretation	78
3.4. Discussion	80
3.5. Limitations of the study	81
Chapter 4 : Early diagnostic performance of Heart-Fatty Acid Binding Protein in suspected Acute Myocardial Infraction	82
4.1 Introduction.....	83
4.2. Aim and Objective.....	87
4.3. Methodology	87
4.3.1. Data collection and processing	87
4.3.2. Measurements.....	88
4.4. Follow up	89
4.5. Outcome	89
4.6. Statistics	89
4.7. Result.....	90
4.7.1. Diagnostic performance of (FABPulous-FABP) as the only biomarker to rule in and rule out AMI on patient arrival and three hours later	90
4.7.2. Diagnostic performance of FABPulous h-FABP True Raid Test and troponin to rule in and rule out) AMI on patient arrival	92

4.7.3. Diagnostic performance of FABPulous h-FABP True Raid Test combine with ECG interpretation and i-STAT troponin result to 'rules in' and 'rules out' AMI strategy	93
4.4. Discussion	95
Chapter 5 : Improved sensitivity of point- of- care troponin I values using reporting the limit of detection and functional sensitivity to rapidly rule out myocardial infarction early results of the Bedside Evaluation of Sensitive Troponin (BEST)	98
5.1. Background.....	99
5.2. Aim and objectives.....	101
5.3. Materials and methods.....	102
5.3.1. Data collection and processing	102
5.3.2. Interventions.....	102
5.3.3. Outcome measuring and defining outcomes	103
5.3.4 Statistical methods.....	104
5.4 Result.....	105
5.4.1. Study population.....	105
5.4.2 Receiver operating characteristic (ROC) curve for the i-STAT troponin assay at presentation, for diagnosis of AMI in the total study population.	105
5.4.3 Performance of the i-STAT POC assay at the limit of detection .02 ng/mL (µg/L) (LoD, 20ng/L)	107
5.4.4 Performance of the i-STAT POC assay at the conventional 99 th percentile cut-off 0.08 ng/mL	110
5.4.5 Performance of i-STAT POC assay at the functional sensitivity (10ng/L) of the assay (0.01 ng/mL).....	112
5.4.6 Diagnostic Accuracy of the Functional sensitivity of i-STAT POC on arrival and 3 hours post arrival with ischemic ECG.....	114
5.4.7 Diagnosing AMI.....	115

5.5	Discussion	116
5.6	Strength of the study.....	122
5.7	Limitation and strength of the study	123
Chapter 6 : Multicentre, prospective validation of the Troponin-only Manchester Acute Coronary Syndromes decision aid using a single point of care troponin test in the Emergency Department.....		124
6.1.	Background.....	125
6.2.	Aim and objectives.....	128
6.3.	Methods.....	128
6.3.1.	Participants	128
6.3.2.	Laboratory analyses and data collection	129
6.3.3.	Application of the T-MACS decision aid with a POC cTn test	129
6.3.4.	Statistical analysis	130
6.3.5.	Follow-up.....	131
6.3.5.	Outcome.....	131
6.4.	Results.....	133
6.11.	ROC curve for T-MACS probability.....	136
6.5.	Discussion	137
6.6.	Conclusions	142
6.7.	Strength and limitation of the study.....	143
6.8.	Future directions	144
References.....		145
Appendices		181
Appendix 1.1		181
Appendix 2.1.....		184
Appendix 2.2.....		186
Appendix 2.3.....		188
Appendix 2.4.....		190

Appendix 2.5	198
--------------------	-----

List of figures

Figure 1-1. Atherosclerosis plaque formation	6
Figure 1-2. Disruption of atherosclerotic lesion results in atherothrombosis ..	7
Figure 1-3. Stages of plaque development	8
Figure 1-4. Myocardial infarction during acute ST-elevation	12
Figure 1-5. The kinetics of release of creatine kinase MB (CKMB) and cardiac troponin.....	29
Figure 1-6. The troponin complex, tropomyosin, actin and myosin that form the contractile unit of the cardiac muscle cell.....	36
Figure 1-7. Troponin Complex Cross-Section	37
Figure 2-1. Chest pain pathway Initial assessment and treatment of suspected ACS in MRI hospital.....	52
Figure 2-2. The sample-processing flowchart in the BEST study.....	56
Figure 2-3. Four steps for the i-STAT troponin test. In the first picture, blood is placed in the i-STAT cartridge.....	63
Figure 2-4. Plasma Separation Device.	66
Figure 2-5. Result interpretation in The FABPulous device	67
Figure 2-6. Positive result in the FABPulous device.	67
Figure 3-1. Release of h-FABP and cardiac troponins from the injured heart into plasma after acute myocardial infarction (AMI)	72
Figure 3-2. A variety of POCT for heart fatty acid binding protein testing	75
Figure 4-1. h-FABP: Release Kinetic.	86
Figure 4-2. The qualitative FABPulous h-FABP	89
Figure 5-1. Receiver operating characteristic (ROC) curve for the i-STAT troponin assay at presentation, for diagnosis of AMI in the total study population	106
Figure 5-2. Receiver operating characteristic (ROC) curve analysis for the i-STAT troponin assay measured at three hours after presentation in the total study population, for a diagnosis of AMI	106
Figure 6-1. A variety of POCT platforms	128
Figure 6-2. Proportion of patients with AMI in each of the T-MACS risk groups	135
Figure 6-3. Receiver operating characteristic curves showing the overall accuracy of T-MACS.....	137

List of tables

Table 1-1. Classification of Myocardial infarction.....	10
Table 1-2. Modifiable and non-modifiable risk factors for coronary heart disease	14
Table 1-3. TIMI Risk Score variables for unstable angina and NSTEMI	19
Table 1-4. The GRACE risk score.....	20
Table 1-5. Troponin-only Manchester Acute Coronary Syndrome decision rule (TMACS) and the Manchester Acute Coronary Syndrome decision rule (MACS) Variables	24
Table 1-6. MACS and T-MACS risk groups	24
Table 1-7. Comparison between MACS and T-MACS rule	25
Table 1-8. Optimal Cardiac Marker: Characteristics	31
Table 1-9. Acute Myocardial Infarction: Plasma Biomarkers Characteristics	31
Table 1-10. Potential Causes of Troponin Level Elevation in Blood, Excluding AMI	38
Table 1-11. Troponin characteristics.....	38
Table 1-12. Potential Utility and Challenges of the High-Sensitivity Troponin Assay	42
Table 1-13. Benefits of Point-of-Care Testing	45
Table 1-14. Barriers to Implementation of Point-of-Care testing in hospitals	45
Table 1-15. Recommended criteria for the prioritisation of POCT.....	46
Table 2-1. Sample collection schedule	55
Table 2-2. Equipment and consumables.....	61
Table 2-3. Clinical characteristics of (i-Stat, Abbott Point of Care).....	64
Table 2-4. Important analytical definitions in the use of troponin assays	64
Table 3-1. The interpretation of Cohen's kappa classification.....	78
Table 3-2. Baseline characteristics of patients recruited to the interobserver reliability study	79
Table 4-1. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the FABPulous h-FABP True Raid Test only decision aid at patient presentation.(n= 691)	91
Table 4-2. Significance of diagnostic Rule-out strategy of FABPulous h-FABP at patient presentation on patient arrival	91

Table 4-3. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the FABPulous h-FABP True Raid Test only decision aid with at three hours post arrival (n= 663).....	92
Table 4-4. Significance of diagnostic Rule-out strategy of FABPulous h-FABP at Three hours post presentation post arrival.....	92
Table 4-5. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for rule-out and rule- in myocardial infarction on arrival and three hours later of patient arrival for h-FABP combined with i-STAT >0.02ng/ml.....	93
Table 4-6. Significance of diagnostic Rule-out strategy of FABPulous h-FABP and i-STAT result on arrival and three hours later.	93
Table 4-7. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival and three hours later of patient presentation	94
Table 4-8. Significance of diagnostic Rule-out strategy of FABPulous h-FABP and ECG interpretation and i-STAT result on arrival and three hours later	94
Table 5-1. Optimal cut off-values of i-STAT at each cut-off value at initial time for AMI with 95% CI	107
Table 5-2. Optimal cut off-values of i-STAT at each cut-off value at three hours later.....	107
Table 5-3. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival using the 0.02 ng/ml cut-off	109
Table 5-4. Rule-out strategy, POC cTn<0.02 µg/L on arrival	109
Table 5-5. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction at three hours later of patient presentation using the 0.02 ng/mL (µg/L) cut-off.....	109
Table 5-6. Rule-out strategy, POC cTn<0.02 ng/mL (µg/L) on arrival & 3 hours post arrival	110
Table 5-7. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in	

myocardial infarction at arrival of patient presentation using the 0.08µg/L the conventional 99th percentile cu.....	110
Table 5-8. Rule-out strategy, POC cTn 0.08µg/L, the conventional 99th percentile cut-off on arrival.....	111
Table 5-9. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival & 3 hours later of patient presentation using the 0.08µg/L cut-off.....	111
Table 5-10. Rule-out strategy, POC cTn 0.08µg/L on arrival & 3 hours post arrival	111
Table 5-11. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival and three hours later of patient presentation using the functional sensitivity.....	112
Table 5-12. Rule-out strategy, POC cTn at the functional sensitivity of the assay <0.01 µg/L on arrival & 3 hours post arrival	112
Table 5-13. Summary of the diagnostic accuracy specification for i-STAT cardiac troponin assay for ruling out AMI at different cut offs	114
Table 5-14. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival and three hours later of patient presentation with the functional sensitivity.....	115
Table 5-15. Rule-out strategy, POC cTn at the functional sensitivity of the assay <0.1 µg/L on arrival & 3 hours	115
Table 6-1. Predictors in the Manchester Acute Coronary Syndromes (MACS) model Variable Measurement scale.....	130
Table 6-2. T-MACS Groups risk probability	131
Table 6-3. Baseline characteristics of included patients	134
Table 6-4. Proportion of patients with AMI in each of the T-MACS risk groups total number of patients including in the analysis 634.....	135
Table 6-5. Prevalence of AMI stratified by T-MACS risk group	136
Table 6-6. Test characteristics of T-MACS (very low risk vs all other risk groups).....	136

Table 6-7. Two x two table showing the prevalence of AMI stratified by T-MACS risk group (high risk vs all other risk groups)	136
Table 6-8. Significance of diagnostic Rule-out strategy of T-MACS for high-risk group only.....	136
Table 6-9. Comparison of the T-MACS with the current protocol.....	141

Abstract

Cardiac troponin is the reference standard biomarker for the diagnosis of acute myocardial infarction (AMI). In the appropriate clinical context, the detection of a rise and/or fall of cardiac troponin is highly sensitive and specific for this diagnosis. However, troponin testing has two key limitations. First, as levels in serum or plasma can take several hours to rise, the diagnostic sensitivity of troponin testing is insufficient to allow acute coronary syndromes to be safely 'ruled out' and serial testing remains necessary. Second, because of the need to detect a rise and/or fall of troponin, serial testing is essential to differentiate chronic troponin elevations from those related to AMI. As a result, international guidance currently recommends serial testing over 6-12 hours. Recent evidence suggests that, with contemporary sensitive troponin assays, AMI can often be 'ruled out' and/or 'ruled in' with serial testing over as little as 1 to 3 hours. However, as the turnaround time of laboratory-based testing is typically 1-2 hours, these results may still be unavailable at the time key decisions about initial treatment and patient disposition are made in the Emergency Department (ED). Point of care troponin (POCT) testing at the patient's bedside has a shorter turnaround time than laboratory-based assays and eliminates the need to transport the sample to a central laboratory. When patient pathways are appropriately designed to accommodate point of care testing, key management decisions may be expedited, potentially reducing ED length of stay. If the accuracy and safety of rapid diagnostic strategies using point of care troponin testing over 3 hours can be demonstrated, there are tremendous potential benefits for ED throughput, healthcare resource use and for patients (both in terms of receiving appropriate reassurance with avoidance of hospital admission and receiving appropriate early treatment for acute coronary syndromes). In this study, we will evaluate several promising strategies that may enable clinicians to make accurate diagnoses based on information available in the ED and a single blood test for cardiac markers at the POC. During the study period of February 2015 to March 2017 there were 1,613 patients enrolled. Of this cohort, some patients were excluded for missing i-Stat on arrival, and the patients who did not have an ECG recorded were excluded. This left 733 patients in the final group for analysis consisting

of 457 men (62.3%) and 276 women (37.7%). The mean age was 58 years (standard deviation 16). In a pragmatic study we determined the interobserver reliability of Heart-type fatty acid-binding protein (h-FABP) the absolute agreement between investigators was 93.0% with a kappa of 0.81 (95% CI 0.6–1.0), indicating near perfect agreement. In total there were three (7.0%) disagreements. The diagnostic accuracy of POC h-FABP lateral flow immunoassay (True Rapid, FABPulous BV) device for diagnosing or excluding AMI using a single test at the time of patient presentation to the ED and three hours later has been evaluated, the sensitivity and NPV to rule out AMI were 48.24% (95%CI: 37.26% to 59.34%) and 92.48 % (95%CI: 90.91% to 93.80%) respectively. While Specificity and PPV were 89.27 % (95%CI: 86.53%to 91.62%) and 38.68% (95%CI: 31.45% to 46.44%) respectively. However, this strategy would allow 85 % of patients to be discharged (rule out percentage). The diagnostic accuracy of a contemporary POC cTn assay used on arrival and 3 hours later in patients with suspected ACS, at the conventional 99th percentile and novel LoD cut-offs was also evaluated. Finally, we were validated the T-MACS with a contemporary POC cTn assay (i-Stat, Abbott Point of Care, New Jersey) in order to investigate the clinical diagnostic accuracy of i-Stat device to rule out AMI in EDs. By setting the cut off levels of POC at 10ng/ml and functional sensitivity at 10% CV, the T-MACS rule has successfully 'ruled out' > 41.8% of patients with suspected cardiac chest pain following a single blood test, with a sensitivity of 97.4% (90.8 - 99.7%) and NPV of 99.3% (97.1 – 99.8%), in a very short turnaround time of 5-10 minutes. On the other hand T-MACS has risk stratified the patients who were at high risk to have AMI with a specificity of 93.56% (91.27% to 95.40%) and a PPV of 50.00% (42.08% to 57.92%). To our knowledge, this is the first successful validation of a single test 'rule out strategy' using a POC cTn assay. With a 5-10 minute turnaround time, this assay could help to unburden crowded EDs by enabling almost immediate reassurance and discharge for >40% of patients with suspected cardiac chest pain.

Declaration

I declare that this thesis is all my own work and has not been copied from any other sources, or accepted for any other degree in any University. To the best of my knowledge, this thesis contains no material written or distributed previously by any other parties, apart from where I have otherwise stated.

List of publications and achievements derived from this project

Malak Al Mashali; Niall Morris; Garry McDowell and Richard Body. The interobserver reliability of a novel qualitative point of care assay for heart-type fatty acid binding protein. Article published in Clinical Biochemistry, 49(15), 2016/10/01/, pp. 1199-1201.

Malak Al Mashali; Niall Morris; Garry McDowell and Richard Body. The interobserver reliability of a novel qualitative point of care assay for heart-type fatty acid binding protein. Poster presented at AACCC's 26th International Point-of-Care Conference, from September 21 through September 24, in Copenhagen.

Malak Al Mashali; Richard Body and Garry McDowell. Rapid acute myocardial infarction rule-out using the limit of detection of a point of care troponin assay: early results of the Bedside Evaluation of Sensitive Troponin (BEST) study. Poster presented at ESC 2017 Congress from Saturday 26 - Wednesday 30 August 2017 in Barcelona, Spain.

Richard Body; Malak Al Mashali and Garry McDowell. Multicentre, prospective validation of the Troponin-only Manchester Acute Coronary Syndromes decision aid using a single point of care troponin test in the Emergency. Poster presented at the European Congress of Emergency Medicine from 23-27 September 2017 in Athens, Greece.

Malak Al Mashali, Richard Body, Garry McDowell Absolute and relative changes (delta) cardiac troponin I using a contemporary point of care assay for early diagnosis of myocardial infarction. Poster presented at ESC 2018 Congress from Saturday 25 to Wednesday 29 August 2018 in Munich – Germany.

Acknowledgements

First and above all, I praise God, the almighty for providing me this opportunity and granting me the capability to proceed successfully. This thesis appears in its current form due to the assistance and guidance of several people. I would therefore like to offer my sincere thanks to all of them.

Initially, I would like to extend my deepest appreciation to my supervisory team my director of studies study Dr Garry McDowell and Professor of Emergency Medicine at Manchester Royal Infirmary Professor Richard Body for their support, encouragement, patience and guidance needed in completing the dissertation process. Their assistance, knowledge, and dedication greatly contributed to my success and helped me preserve through the hard times and celebrate the good times. I am grateful for the support they offered me during the whole period of the study.

I would like to thank my colleagues at the emergency & intensive care research group and clinical research nurses for their help during the recruitment journey at Manchester Royal Infirmary, special thanks to Sarah Douglas for helping me in contacting the participant sites and collecting the missing information needed to completed this study without whom this research would simply not have been possible.

I would also like to thank my colleagues at the School of Healthcare Science and special thanks to Glenn Ferris for his supports and help during my study.

This work could not have been completed without constant encouragement and support from my family I am especially grateful to my parents, who supported me emotionally during the whole journey I owe my success to you I love you both and cannot express how important your presence has been throughout my writing time. Mother, Nahid Attiyah, you have played an important role in the development of my identity and shaping the individual that I am today, I am so lucky to have you.

Special thanks to my brothers and sisters for the love, prayers, support, encouragement, and guidance, I may not have made it to the end. Thank you and God bless you all.

To my many friends you should know that your support and encouragement was worth more than I can express on paper. Special thanks to my friend Manal, thank you for, offering me advice, and supporting me through this entire process. Alaa, I cannot fully express how much I appreciate your listening and support.

Finally, I would like to extend my greatest thanks to my sponsor Prince Sultan Military Medical City. Thank you for the financial and moral support throughout my study.

I am sure I have forgotten to name someone who was crucial to my success, please forgive me, but know that in my heart I am eternally grateful.

List of Abbreviations

CAD	Cardiovascular disease
ACC	The American College of Cardiology
ACS	Acute Coronary Syndrome
ADP	Accelerated Diagnostic Protocol
AMI	Acute Myocardial Infarction
ASPECT	Asia-Pacific Evaluation
BHF	British Heart Foundation
CPRD	Clinical Practice Research Datalink
CPU	Chest pain unit
CRF	Case Report Form
cTn	cardiac troponin
ED	Emergency department
ESC	European Society of Cardiology
hs-cTnT	high sensitivity cardiac troponin T
LBBB	left bundle branch block
MACS	The Manchester Acute Coronary Syndromes
NHS	the National Health Service
NICE	The National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
POCT	Point of care testing
ROC	Receiver Operating Characteristic
TAT	Turnaround time
TMACS	Troponin-only Manchester Acute Coronary Syndromes
UA	unstable angina
UK	United Kingdom

Chapter 1 : Introduction

1.1. Background

Chest pain is the most frequent symptom presented in Emergency Departments (ED). Cardiovascular disease (CVD) remains the leading cause of mortality globally, and accounts for about one-third of all deaths. About 114,000 patients with acute coronary syndromes (ACS) are admitted to hospital each year in United Kingdom (Souter, 2014). Several underlying pathophysiological processes might be a reason for chest pain, but the most common, serious cause is ACS. ACS refers to a set of signs and symptoms that occur when the blood supplied to the heart muscle is suddenly blocked. This symptom combines the clinical conditions of acute myocardial infarction (AMI) and unstable angina (UA) (Timmis, 2015). A recent report released by the British Heart Foundation (BHF) on Cardiovascular Disease Statistics, reported that cardiovascular disease was the cause of death in 27.4% of men and 25.2% of women (BHF, 2017). Patients with symptoms suggestive of AMI account for about 10% of all (ED) consultations in the United Kingdom (UK). By 2030, it is expected that 23.3 million will die annually from cardiovascular disease (Peters *et al.*, 2007; World Health, 2014). From updated trends using data from the national statistics agencies of the United Kingdom, coronary heart disease by itself is still the single most significant cause of death with increases in treatment and hospital admissions for all CVD patients (Bhatnagar *et al.*, 2016). Between 2010/2011 and 2013/2014, hospital admissions for all CVD patients increased by over 46,000 with more than 36,500 of these increased admissions for men (Bhatnagar *et al.*, 2016).

Patients presenting with chest pain account for 5% to 10% of all visits to emergency departments (ED). Approximately one-third of attendances and half of the admissions had a clinical diagnosis of ACS without clear ECG changes (Goodacre *et al.*, 2005). In the UK, a Clinical Practice Research Datalink (CPRD) database reports that more than 915,000 people suffer from AMI and more than 1.3 million are living with angina (Bhatnagar *et al.*, 2015). A recent report by the BHF, suggests that CVD's cost to the UK economy is estimated to be over £15 billion each year, and the healthcare costs relating to CVD are estimated to be about £11 billion each year. Conversely, in the US in 2011, the cost of admitting patients with chest pain totalled \$11.5 billion,

which represents 3% of that nation's healthcare expenses and \$11,504 of the aggregate hospital (Torio and Andrews, 2006). On the other hand, the pressure to find hospital beds in the National Health Service (NHS) in England and Wales is well known. It has been reported that the number of patients ready to be discharged medically, but still occupying a medical bed, has increased significantly. According to a report from Delayed Transfers of Care Data, up to July 2017, there were 118,100 patients delayed in acute care.

These so-called "delayed transfers" are a crucial driver for high bed-occupancy rates within hospitals. On the other hand, emergency departments are under enormous pressure to meet the 4-hour target (<4 hours from patient presentation to discharge or transfer), which is a key metric by which NHS Trusts are evaluated and accordingly compensated or disciplined. As a result of this challenge, there has been considerable interest in research on rapid decision-making as well as extensive interest in developing a single test, available acutely, to discharge patients in EDs safely. The prompt and accurate evaluation of acute chest pain has immense implications for patient morbidity and mortality, and healthcare economics overall. In contrast, identifying patients with chest pain who are at risk of adverse events is crucial, as the mortality rate of patients with missed AMI is at least double that of similar patients who are accurately *diagnosed* (Pope *et al.*, 2000). *However*, this is important not only to ED physicians, but also to all physicians who evaluate chest pain patients.

Only 10% to 20% of patients, who are admitted to hospital with the symptoms of chest pain, are diagnosed with MI; most patients are diagnosed with non-cardiac conditions and 10% of women experience no chest pain at all during a heart attack (Dey *et al.*, 2009). Most patients examined for ACS do not have this condition, and previous studies report up to 90% of all patients investigated have normal findings (Hollander, 1999; Pope *et al.*, 2000). Based on latest statistics report in 2015, cardiac heart disease (CHD) is responsible for nearly 70,000 deaths in the UK each year, an average of 190 people each day, or one death around every eight minutes (BHF, 2015). The diagnosis (rule in) of ACS is based on clinical assessment, which includes electrocardiograph (ECG) and cardiac troponin (cTn) measurements. The challenges for ED

physicians in evaluating chest pain in patients is not only to rapidly rule in ACS, but also to rule out this serious condition that may cause morbidity and mortality. However, to meet this challenge, numerous diagnostic procedures have been investigated during the past two decades, including new cardiac biomarkers, early stress testing, clinical risk scores and non-invasive imaging of the myocardium and coronary arteries (Kontos *et al.*, 2010). These have been incorporated into various accelerated diagnostic protocols (ADPs) or chest pain unit (CPU) pathways to provide a rapid, cost-effective mechanism for evaluation. It has been reported that only a quarter of all patients (25%) who are admitted on suspicion of ACS have AMI (R. Body *et al.*, 2014c). Most of these protocols are based on laboratory testing, and this poses a question. Can a Point-of-Care Troponin I Assay be as good as a central laboratory assay, and become an alternative diagnostic method to diagnose AMI in EDs?

1.2. The pathophysiology of coronary heart disease

Coronary artery disease (CAD) is most commonly caused by atherosclerosis (Hobbs and Arroll, 2009), which is developed when plaque builds up in the artery walls from low-density lipoproteins (LDL). The (ACS) refers to any group of clinical symptoms resulting from acute reduction in blood flow to the heart. ACS includes both chest pain at rest (unstable angina) and Acute Myocardial Infarction (AMI) (Kumar and Cannon, 2009). Figure 1.1 shows how the infarct-related coronary artery can experience complete or partial thrombosis as a result of atherosclerotic plaque rupture, which is associated with ACS (Weissberg, 1999). Before patients experience this acute event, it can take several decades for processes of atherosclerosis to progress and develop from syndromes that display pathophysiologic mechanisms that often remain hidden.

Plaque is formed on arterial walls due to fibroblasts, smooth muscle cells and cholesterol accumulation, which cause these arterial walls to narrow and thicken. Subsequently, the circulation of blood becomes blocked or slowed, as plaque accumulation increases, but the blood vessels also experience constriction caused by endothelial dysfunction before the formation of the plaque (Ambrose and Singh, 2015). Risk factors include genetic causes, diets that are high in cholesterol and saturated fat, obesity, diabetes, hypertension, smoking, and LDL and other lipoprotein abnormalities known as dyslipidaemia that are widely reported, which contribute to medium sized arteries inner lining or intima that become increased by its low-grade inflammatory state (Siri-Tarino *et al.*, 2010). Figure 1.2 shows how coronary atherosclerosis contributes to the lumen of the artery narrowing over time to various degrees, so that the coronary arteries' inner layers are subjected to thickening in a progression that is slow (Lilly and Harvard Medical, 2016). The flow of blood in the artery is often changed at arterial bifurcation points, so that sudden cardiac death (SCD) and AMI caused by atherosclerosis is shown to prefer proximal segments of major coronary arteries (J. C. Wang *et al.*, 2004). There are two processes where cycles of rapid progression could interrupt the slow progress of atherosclerosis: involving plaque haemorrhage, or when a non-

occlusive intraluminal thrombus is formed with asymptomatic plaque disruption. Stages of plaque development are illustrated in Figure 1.3

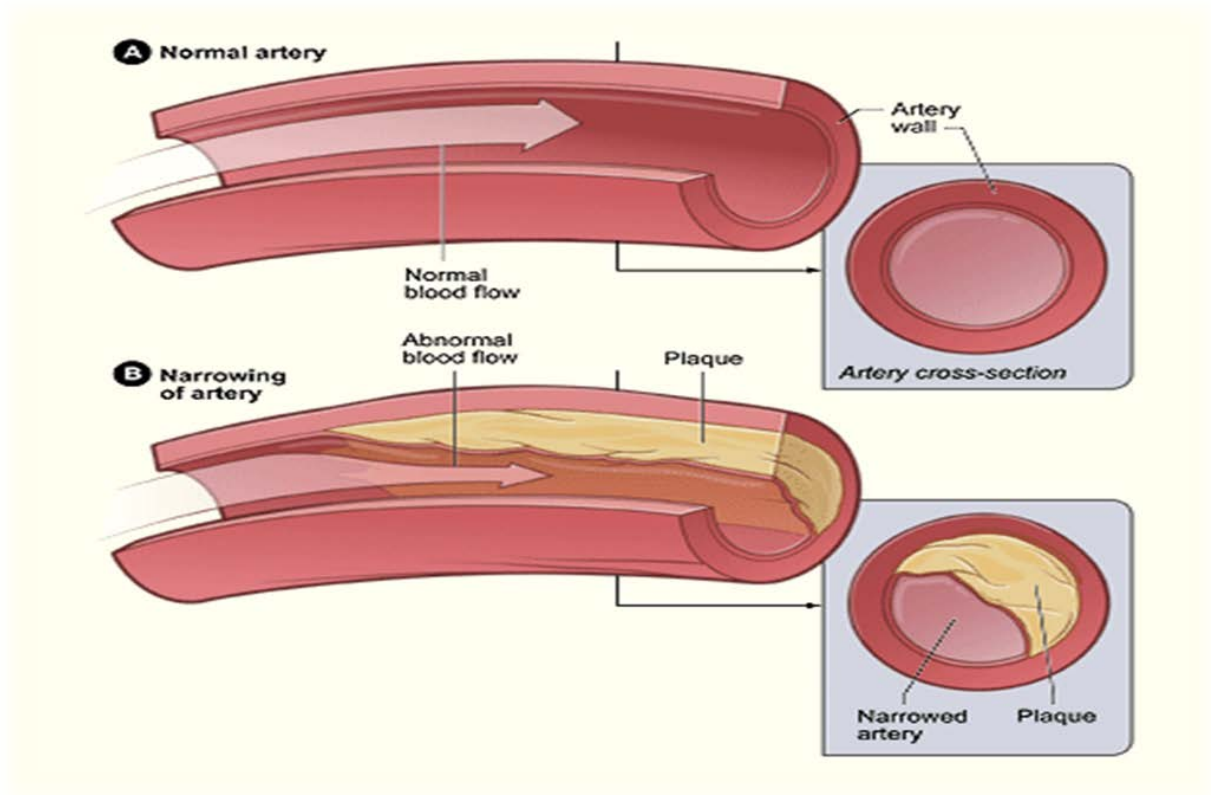


Figure 1-1. Atherosclerosis plaque formation

A. Normal artery with normal blood flow. The additional image shows a cross-section of a normal artery. **B.** An artery with plaque build-up. The additional image shows a cross-section of an artery with plaque build-up (taken from (NIH, April 25, 2013)).

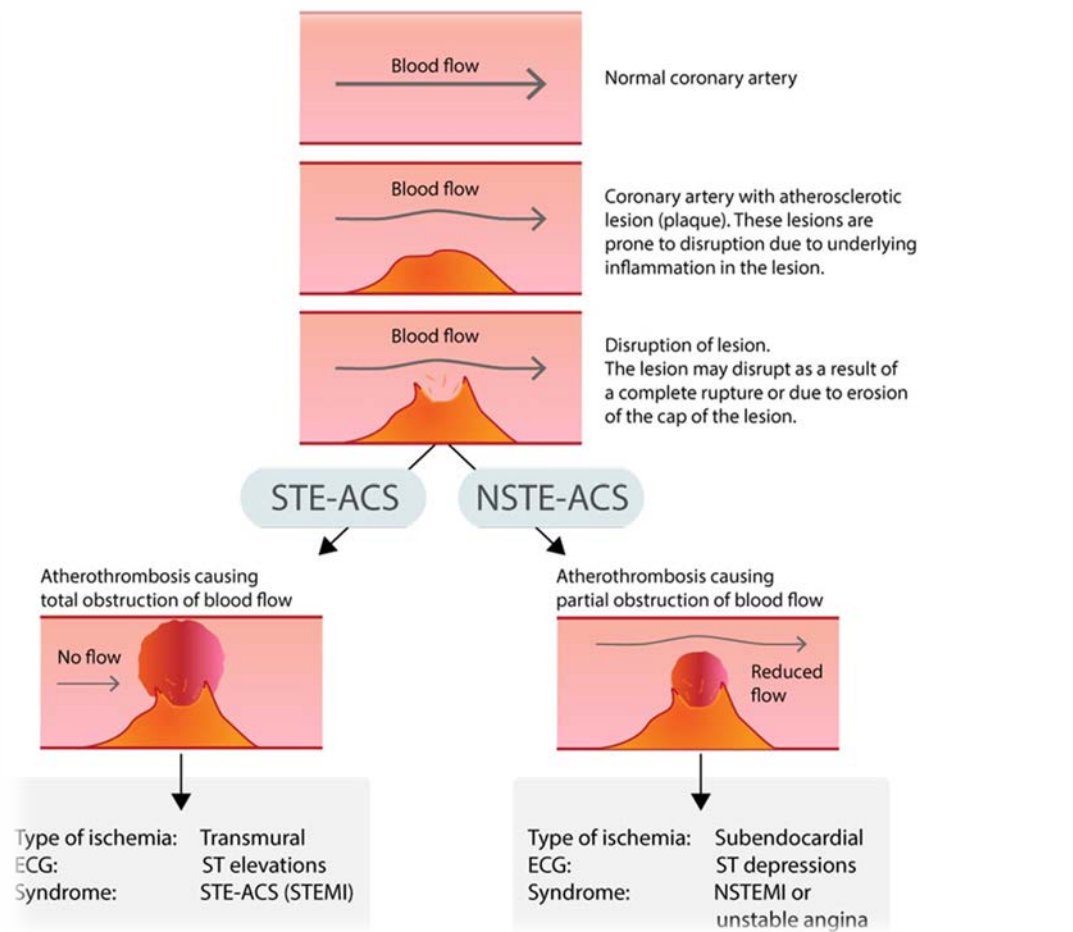


Figure 1-2. Disruption of atherosclerotic lesion results in atherothrombosis

This leads to an abrupt reduction in coronary blood flow. STE-ACS (STEMI) occurs if the occlusion is complete. On the other hand, STE-ACS (NSTEMI and unstable angina) occurs if the occlusion is partial.

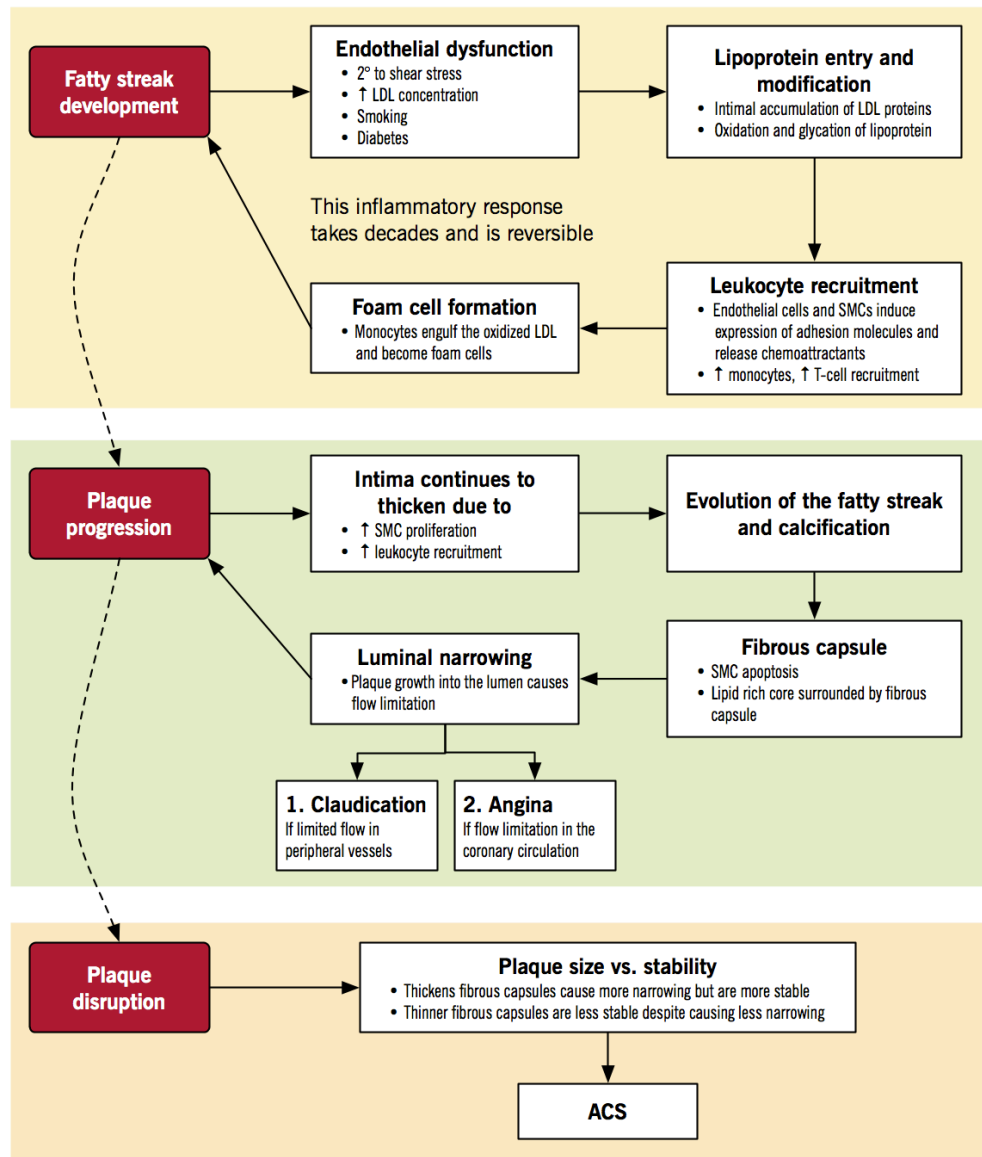


Figure 1-3. Stages of plaque development

1.3. Myocardial infarction

Myocardial injury frequently occurs in patients without ACS. The development of a myocardial infarction begins with the initial onset of myocardial ischemia, which comes from an imbalance of oxygen supply and demand, which leads to myocardial cell damage or death (Ambrose and Singh, 2015). The four-decade-old World Health Organisation (WHO) definition of AMI has been replaced by clinical criteria. This definition, established by the European Society of Cardiology (ESC) and American College of Cardiology focuses mainly on defining infarction as evidence of myocardial necrosis. This definition refers to the Third Universal Definition of myocardial infarction (MI) published in October 2012 by the Global Myocardial Infarction Task Force (Thygesen *et al.*, 2012a). In the diagnosis, this definition requires cardiac myocyte necrosis with an increase and/or decrease in plasma of cardiac troponin (cTn). This requires at least one value to be above the 99th percentile normal reference limit in addition to evidence of myocardial ischemia from symptoms, significant electrocardiogram (ECG) changes, or cardiac imaging, or other evidence showing a new wall motion abnormality in a segment of the myocardium. International guidelines recommend patients with myocardial infarction to be classified by etiology (Roffi *et al.*, 2015). AMI is categorised according to the underlying mechanism. The acute myocardial injury is classified where troponin concentrations are elevated with evidence of dynamic change in the absence of apparent myocardial ischemia, whereas in chronic myocardial injury troponin concentrations remain unchanged on serial testing (Chapman *et al.*, 2016).

Table 1-1. Classification of Myocardial infarction

Type	Mechanism
1	Spontaneous MI caused by atherosclerotic plaque rupture. A thrombus will block one or more of the coronary arteries leading to decreased myocardial blood flow and ischemia
2	MI secondary to myocardial oxygen supply and demand imbalance, caused by a condition other than atherosclerotic disease, such as coronary artery spasm, sepsis, arrhythmias, respiratory failure, hypotension or hypertension
3	MI caused by presumed myocardial ischemia leading to cardiac death before biomarkers are obtained or before the biomarker appears in the blood.
4a	MI related to coronary angiography or PCI
4b	MI associated with stent thrombus as diagnosed by angiography or autopsy
5	MI associated with CABG

Abbreviations: myocardial infarction (MI), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG)

1.3.1. ECG: AMI classification

ECG tests are immediately given to ED patients that present with chest pain in hospital and clinical settings, and Figure 1.4 shows an abnormal record and a normal record. STEMI is one form of myocardial infarction associated with pathologic Q waves, but this cannot provide differentiation between MI that presented years or weeks earlier and an acute event. However, when the T wave shows a peaked appearance and when the ST segment is elevated, a distinction can be observed when T-wave abnormalities and ST abnormalities in a temporal sequence are produced by an acute STEMI. Q-waves have not developed and myocardial cells remain viable at this early stage, so that ST segments can return to baseline when treatment is given to patients to restore blood flow with acute coronary reperfusion. In contrast, when acute coronary reperfusion is not successful for patients, the ECG shows the R wave has a loss of amplitude and a pathologic Q wave is inscribed and positioned above the infarction territory. The Q wave deepens, the T wave inverts and ST segments remain elevated around 2 days after infarction, and after more days, the T wave is still inverted but the ST segment elevation changes to baseline. When a period of months or weeks has elapsed after the infarct, the T wave and ST segment are shown to be normal, but MI produces a permanent marker of persistent pathological Q waves, and at the site of infarction, a bulging fibrotic scar often develops, when after several weeks the ST segment is still elevated. The leads that overlie the infarction zone record the T wave, ST and QRS evolutionary changes (Lilly and Harvard Medical, 2016) (see Figure 1.4).

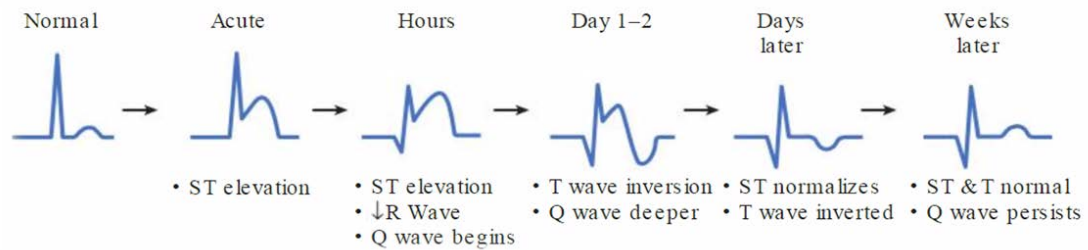


Figure 1-4. Myocardial infarction during acute ST-elevation

ECG evaluation during acute ST elevation myocardial infarction (also termed acute Q wave myocardial infarction) if successful early reperfusion of coronary occlusion is achieved the elevated ST segments return to baseline without subsequent T wave inversion or Q wave development. (Adapted from (Lilly and Harvard Medical, 2016))

The first concern for emergency physicians is whether the patient presents an ST-segment elevation myocardial infarction (STEMI), where the ST-segment signifies the interval between ventricular depolarisation and the start of repolarisation (Lux, 2017). Patients with suspected STEMI should receive an ECG within 10 minutes of arrival at a hospital, remain on continuous electrocardiogram monitoring, and have blood drawn for cardiac biomarkers (Steg *et al.*, 2012). When an atherosclerotic plaque in the artery wall ruptures, it could be the main reason for total artery occlusion, causing ischemia in the myocardium supplied by that artery (Hanna and Glancy, 2015). The National Institute for Health and Clinical Excellence (NICE) has recently issued guidelines for physicians (Cooper *et al.*, 2010).

Patients with STEMI have a well-established treatment path, including drugs or percutaneous coronary intervention (PCI) (Damman *et al.*, 2017), and these patients should rapidly undergo either coronary angiography or thrombolysis. Conversely, in UA and NSTEMI, a disrupted atherosclerotic plaque progresses into a non-occlusive thrombus, which causes a 15% narrowing of the coronary artery, decreased myocardial perfusion, and can present as a transient or persistent ST segment depression or T wave inversion, flattened T waves, pseudo normalisation of T waves, or no changes on the electrocardiogram (Amsterdam *et al.*, 2014; Hamm *et al.*, 2011). The ECG may show ST segment depression or T wave changes. In the case of partial coronary artery occlusion, an earlier onset of sub endocardial repolarisation occurs leading to a depression of the ST segment, and a reverse

electrophysiological wave results in an inverted T wave. However, in this case the main goal is to stabilise patients' pain using medication for blood-thinning agents, such as aspirin and heparin, because these have been proven to improve outcomes for patients with NSTEMI (Kumar and Cannon, 2009). During NSTEMI, the primary goal is to stabilise the patient using nitrates and morphine for the chest pain, beta-blockers, platelet inhibitors and anticoagulants. Thereafter, the physician decides on conservative therapy or invasive methods, such as coronary angiography with the possibility of revascularisation (Jokhadar and Wenger, 2009).

Biomarker evaluation, risk stratification and responses to prescribed therapy for the basis for clinical decisions, and NSTEMI and unstable angina are mostly similar, except that biomarkers for unstable angina are not increased, but the same treatment is given for both conditions. Comparisons of patients with type 1 AMI and type 2 AMI have been investigated in many studies to reveal differences in patient outcomes and differences in diagnosis. One recent study reports that type 2 AMI was present in 14% of cases and type 1 AMI was shown in 86% of cases (Lopez-Cuenca *et al.*, 2016). When investigating all MIs in patients admitted to hospital, the most recent study reports that 88.5% of patients presented type 1 AMI, while (7.1%) were classified as type 2 AMI (Radovanovic *et al.*, 2017). Type 3 MI is uncommon, and found in approximately 3% to 4% among all types of AMI (Jangaard *et al.*, 2017).

1.4. Acute coronary syndrome assessment and management

1.4.1. Risk factors for acute coronary syndrome

The likelihood of developing coronary heart disease is generally determined by various risk factors, and Table 1.2 shows that these can be non-modifiable or modifiable, but changes in lifestyle are proven to reduce many of these modifiable risk factors (Huma *et al.*, 2012). MI long-term risks are increased by these factors, but for patients in EDs presenting with chest pain symptoms, the ongoing probability of MI is not necessarily increased. In one study, the influence of risk factors for CHD was investigated, and it reported 21.3% of patients with 4 or 5 risk factors had AMI, but 12.2% of patients who presented no cardiac risk factors had AMI (Body *et al.*, 2008). Sudden cardiac death, premature atherosclerosis and myocardial infarction are mostly caused by patients smoking, which is a strong risk factor, and compared with patients with healthier lifestyles; early STEMI is caused in patients who smoke. Therefore, smoking doubles the chances of CHD compared with non-smoking individuals, and patients who smoke are likely to develop heart disease around seven years earlier than individuals with healthier lifestyles (Zhang *et al.*, 2010).

Table 1-2. Modifiable and non-modifiable risk factors for coronary heart disease

Modifiable risk factors	Non-modifiable risk factors
<ul style="list-style-type: none">• Diabetes• Diet (lack of fruit and vegetables)• Hypercholesterolaemia• Hypertension• Lack of exercise• Obesity• Smoking and alcohol use• Stress	<ul style="list-style-type: none">• Age• Ethnicity• Gender• Genetic factors, for example family history in a first-degree relative at premature age

Adapted from (BHF, 2011)

1.4.2. The use of risk scores for stratification of acute coronary syndrome patients

Clinicians need to decide which patients can be discharged and sent home, and which patients need further tests and treatment, so it is important to accurately measure the levels of risks that chest pain patients present with techniques and tools that are objective. Therefore, clinicians need to avoid discharging patients with adverse cardiac events that are short-term by using specific and sensitive risk-level techniques and tools that are quick and cost-effective, so that patients with chest pain that have a low risk of heart disease can be safely discharged from EDs. Early risk stratification plays a crucial role in the optimal management of non-ST-elevation ACS. However, the challenge is not only to identify high-risk patients, but also to identify patients with low to very low risk with the absence of disease, so that these patients are discharged immediately with minimal testing or interventions (Backus *et al.*, 2011).

International cardiac guidelines state that chest pain patients presenting to the ED should be assessed with a risk stratification tool or risk score (Roffi *et al.*, 2016; Thygesen *et al.*, 2013). Over the past two decades, several clinical prediction rules and scoring systems have been established. These multivariable prognostic models were derived from clinical trial databases or specific subgroups of chest pain (CP) in EDs. Ideally, a clinical scoring system is developed as an effective risk stratification tool that guides clinicians during their evaluation and improves the standardisation of clinical management. However, few of these are used in practice. Risk scores combine and evaluate various predictors to calculate the risk of ACS for individual patients. They are based on clinical information collected during the initial diagnosis of chest pain patients.

Recently, numerous studies have shown increased identification of low-risk patients when combining a risk score with troponin measurements ((Body *et al.*, 2015a; E. W. Carlton *et al.*, 2015b; Halpern *et al.*, 2013; Six *et al.*, 2013). A large amount of literature has been published on the evaluation of risk scores in addition to comparing the discriminatory performance of each score

in clinical practice (Van Den Berg and Body, 2017; Burkett *et al.*, 2014; Khalill *et al.*, 2009). However, the implementation of scoring systems in clinical practice and their current use has some limitations that are addressed in several studies (Engel *et al.*, 2014; Poldervaart *et al.*, 2017; Long, 2016).

1.4.3. Decision aids derived in patients with ACS

1.4.3.1. The Thrombolysis in Myocardial Infarction (TIMI) Score

The Thrombolysis in Myocardial Infarction (TIMI) risk score is a 7-item tool derived from trials of patients with confirmed ACS. The score is used to define the likelihood of ischemic events or mortality in patients with UA or non-ST segment elevation myocardial infarction (NSTEMI). The scoring model helps to divide the patients into low (score 0-2), intermediate (score 3-4) and high (score 5-7) risk categories. The score predicts the risk of all causes of mortality, MI and critical repetitive ischemia needing urgent revascularisation within 14 days after admission. The initial study showed that 4.7% of patients with a score of 0 or 1 had adverse outcomes within 14 days (Antman *et al.*, 2000). Validation studies showed 1.7 to 2.1% of patients with a score of 0 still had adverse outcomes within 30 days (Chase *et al.*, 2006; Scirica *et al.*, 2002). Recently, several trials have addressed the utility of the TIMI risk score in ED patients with undifferentiated chest pain in a risk stratification and patient decision aid for early discharge from the ED.

ASPECT is the name given to the Asia-Pacific Evaluation of Chest Pain Trial to identify ED patients presenting chest pain who are undifferentiated, and is an early example of risk stratification techniques (Than *et al.*, 2011b). The intention was to identify patients that could be discharged early who were identified as a low-risk group by using a biomarker panel point-of-care test with myoglobin, creatine kinase MB (CK-MB) and troponin, ECG and TIMI score at first presentation and then two hours later, in a study of 14 EDs in Pacific and Asian countries. This study applied the following end points for investigation, which were AMI, high-degree atrioventricular block requiring intervention, ventricular arrhythmia requiring intervention, cardiogenic shock, emergency revascularisation, cardiac arrest and death. This investigated a sample population of 3,582 patients, and those that met one of the end-points

mentioned were reported to be 11.8%. This study reports that biomarker panels with no myocardial injury evidence were supported by a rate-rise on repeat testing and predefined cut-offs for single measures, non-ischemic ECG and a TIMI score of 0 were low risk, which represented 9.8% of patients, or 352. Only 0.9%, or 3, had adverse events within these patients at low risk. The study reported a NPV of 99.1% (CI: 97.3%–99.8%), specificity of 11% (CI: 10.0%–12.2%) and confidence interval [CI]: 97.9%–99.8%), which is reported to achieve a sensitivity of 99.3%.

Early discharge for patients is feasible when low-risk groups are identified and risk stratification decision tools are used, which is demonstrated in this research. According to Than (Than *et al.*, 2011b) patients could be discharged early by using the biomarker that uses troponin and a decision tool that is similar to ASPECT, based on findings that more sensitive troponin assays have become more widely available. Another study, known as ADAPT, adopted observation as a methodology to investigate chest pain symptoms presented by patients at EDs, as well as other patients defined at high risk of ACS by perceptions of physicians in the study (Than *et al.*, 2012). It confirmed that these meet ASPECT primary outcomes as a composite, where the risk stratification tool was adopted for patients at three intervals. At presentation, this tool included troponin testing, non-ischemic ECG and a TIMI score of 0, which was repeated after 2 hours, and then again after 30 days for a cohort, and reported an overall rate of adverse outcomes of 15.3% after 30 days.

When this study compared its results with those of ASPECT, it reported that the number of patients identified for discharge from the ED after 2 hours, because they presented as low risk from the results, was significantly increased to 20%, compared with the results reported when adopting ASPECT that were 9.8%. This study also reported that one patient defined as low risk experienced myocardial infarction who then was given revascularisation, as an adverse event.

These researchers report 99.7% NPV (CI: 98.6%–100.0%) and 99.7% sensitivity (CI: 98.1%–99.9%) for this tool, and based on the ADP or accelerated diagnostic protocol, the rates for low-risk patients and high-risk patients were reviewed in terms of interventions, treatment and follow-up testing.

Around 75% of low-risk patients are subjected to stress tests and significant other investigations in current routine medical care. Sensitivity of ADP for MACE could explain the greater sensitivity reported in this study, as 18.3% received additional treatment. Another study in the Asia-Pacific region reports that an acceptable miss rate is possible for short ED time periods when patients can be safely discharged, which attempted to validate earlier studies (Than *et al.*, 2014a). Another study by Cullen (Cullen *et al.*, 2013) in Europe and the Asia-Pacific region reported similar results when using a TIMI score of 0 or 1 and high-sensitivity troponin I measures within a modified ADAPT ADP. However, a lower rate of early discharge of 8.3% compared with routine care was reported in a randomised trial study of ADAPT, which classified patients as not low risk when presenting two chest pain episodes within a 24 hour period or those that used aspirin, and when adopting the TIMI score, this could produce limitations for trial results (Than *et al.*, 2014a).

1.4.3.2. The Modified TIMI Score

The modified TIMI score (Jaffery *et al.*, 2007) is abbreviated for ease of use in ED settings. Unlike the TIMI risk score, the modified TIMI score utilises only four variables: elevated cardiac marker, ischemic ECG changes, age >65 years, and history of coronary artery disease. In a retrospective study of 947 ED patients with concern for ACS, the modified TIMI had a similar predictive performance compared to the original TIMI risk score. Based on these findings, the authors indicated that their simpler modified score might be more useful in the ED setting than the original TIMI risk score. However, the modified TIMI risk score also suffers from the same disadvantages as the original TIMI risk score, with a 2.4% MACE rate even in the lowest risk category and thus is not sensitive enough to be used in isolation as a decision aid to determine which patients are appropriate for early discharge. See Table 1.3 for score variables.

Table 1-3. TIMI Risk Score variables for unstable angina and NSTEMI

Criteria	Value
Age ≥65	+1
≥3 CAD risk factors*	+1
Known CAD (stenosis ≥50%)	+1
Use of aspirin in past 7 days	+1
Severe angina (≥2 episodes in 24 hrs) or persisting discomfort	+1
ST segment deviation of ≥0.05 mV on initial ECG	+1
Positive cardiac marker	+1
Total score	
Low risk	1-88
Intermediate risk	89-118
High risk	≥119

(CAD): Coronary artery disease. *Risk factors for CAD: Family history of CAD, hypertension, hypercholesterolaemia, diabetes, or current smoker. Adapted with modification from (Antman *et al.*, 2000).

1.4.3.3. The Global Registry of Acute Cardiac Events (GRACE) Score

This GRACE score is reported to be similar to the TIMI score from a study of a large sample population of patients who were confirmed to have ACS (GRACE, 2001). Two distinct risk stratification scores were developed from this large data set in this derivation study, as these represented 14 different countries and used observational data to record outcomes and characteristics of patients with ACS. Eight variables were developed to give predictions for in-hospital mortality by Granger *et al.* (Granger *et al.*, 2003) and score variables are shown in Table 1.4, where the predictive value of each item was used to give a weighted score. This study also used derivation cohorts to validate these scores (Granger *et al.*, 2003) and mortality was predicted at excellent levels by the GRACE scores, so that patients with scores ≥ 250 had $>50\%$ risk of mortality, and those with scores of ≤ 100 had $\leq 0.8\%$ risk of in-hospital mortality (see Table 1.4).

Table 1-4. The GRACE risk score

GRACE score									
Age	Points	HR	Points	SBP	Points	Cr	Points	Killip class	Points
<39	0	<70	0	<80	40	0.0–0.39	1	I	0
40-49	18	70-89	5	80-99	37	0.4–0.79	4	II	15
50-59	36	90-109	10	100-119	30	0.8–1.19	7	III	29
60-69	55	110-149	17	120-139	23	1.2–1.59	10	IV	44
70-79	73	150-199	26	140-159	17	1.6–1.99	13	Cardiac arrest	30
80-89	91	≥ 200	34	160-199	7	2.0–3.99	21	Elevated cardiac markers	13
>90	100	-	-	≥ 200	0	≥ 4	28	ST segment deviation	17
Low risk								1-88	
Intermediate risk								89-118	
High risk								≥ 119	

GRACE: Global Registry of Acute Coronary Events HR: heart rate, SBP: systolic blood pressure, Cr: creatinine, Killip classification: I: no clinical sign of CHF II: Presence of rales (crackles) in the lungs, raised jugular venous pressure, or third heart sound (S3 gallop). III: Acute pulmonary oedema, IV: Cardiogenic shock. Adapted with modification from (Alley and Mahler, 2015).

1.4.3.4. HEART Score

The HEART (history, ECG, age, risk factors and troponin) Score was recently developed for chest pain patients presenting to the ED (Six *et al.*, 2008; Backus *et al.*, 2013). The algorithm was not established on multivariate regression analysis, but was developed based on clinical decision experience in patients with known CAD with high a probability of having ACS. These studies reported a short-term MACE rate (AMI or death) between 0.6% and 1.4%. The HEART score is different from TIMI Risk score or GRACE, because the HEART score was designed to predict the likelihood of ACS in patients presenting to the ED with acute chest pain. Instead, TIMI and GRACE are used to risk-stratify patients who have been diagnosed with ACS. By giving each of the five previous variables a score of zero, 1 or 2, each patient will have a score of zero to ten. However, the score divides patients into a low-risk group from zero to three, intermediate risk group from four to six or high-risk groups from seven to ten. The HEART score divides patients into a low-risk group (0-3) and this score supports early discharge of patients. Intermediate discharge (4-6) are the patients who have a risk of 20.3% for an adverse outcome and should be admitted for further clinical observation, and finally, high-risk groups (7-10) are patients with a 72.7% risk, and these patients would require immediate, early treatment including invasive strategies without preceding non-invasive testing. The HEART score was designed to predict the likelihood of ACS in patients presenting to the ED with acute chest pain, when TIMI and GRACE were used to risk stratify patients who had been diagnosed with ACS. Recently, Mahler *et al.* evaluated 282 patients randomised to the HEART Pathway. Researchers confirmed a proven impact on reducing time for patient discharge from the ED. In the study, patients spent significantly less time in the hospital when compared with standard procedures of care. The time of early discharge in the HEART algorithm was 21% higher than the usual care group, 9.9 hours versus 21.9 hours respectively, with no adverse events occurring at 30 days in both groups (Mahler *et al.*, 2015). The pathway has also been tested on secondary analysis using a contemporary sensitive assay and high sensitivity troponin assay. The researchers found no difference in the score of the HEART Pathway between the two assays with both achieving

100% sensitivity and NPV (Mahler *et al.*, 2017). Another study has reported proof that using the HEART Pathway as a decision aid for patients is cost-effective (Riley *et al.*, 2017).

1.4.3.5. MACS and T-MACS

The Manchester Acute Coronary Syndromes (MACS) decision rule and the Troponin-only Manchester Acute Coronary Syndrome decision rule (TMACS) were applied in a computer model, and were derived by multivariate analysis to identify patients with acute coronary syndromes in the ED in 2014 by Body *et al.* These decision rules take account of eight variables, which are recorded or measured at the time of arrival in the ED (Body *et al.*, 2014c). See Table 1.5 for MACS and T-MACS algorithm variables and for the rule formula. The estimated probability of ACS, based on clinical judgment, is described in Table 1.6. A patient arrives at the ED complaining of chest pain suspected to have a cardiac aetiology. A single serum blood test is taken from the patient and the risk of ACS is calculated using the (TMACS) or (MACS) formula. This calculates the probability that the patient will experience a major adverse cardiac event (MACE) within the next 30 days. Based on that estimated probability, the rule assigns the patient to one of four risk groups: very low risk (ACS 'ruled out'; suitable for immediate discharge); low risk (suitable for serial troponin testing in a low dependency environment such as an ED observation unit); moderate risk (requires admission to an Acute Medical Unit); and high risk ('ruled in' and therefore requires admission to a cardiologist). However, by applying the rule in the ED, the sensitivity of MACS for a MACE within 30 days ranged from 100-97.9%. The study reporting the lowest sensitivity stated that the two coronary stenoses (accounting for all the missed MACE) in the very low risk group did not require intervention. Applying the MACS rule could avoid unnecessary hospital admission for over one quarter of patients based on the results of a single blood test, while also immediately identifying 10% of patients as being at high risk (with over 95% positive predictive value). Earlier in 2014, in the original work of the research group, the h-FABP test in the MACS rule was a laboratory-based h-FABP assay using a commercially available ELISA

(enzyme-linked immunosorbent assay) kit. ELISA tests take several hours to be completed and this disadvantage makes the test inconvenient to run in emergency settings with a sufficiently short turnaround time. However, researchers then recalibrated the MACS rule to include a new, automated, commercially available immune turbidimetric assay suitable for quantitative measurement of h-FABP in serum and plasma in blood, which makes the assay appropriate to be used in emergency settings within faster turned around time, and makes it possible to influence clinical decisions within the required timescale for patients attending the ED (Body *et al.*, 2015a). In this study, the researchers included 456 patients, where 78 (17.1%) had AMI and 97 (21.3%) developed a MACE. However, in comparison with the original MACS rule (including the ELISA assay for h-FABP), the recalibrated MACS rule applies the automated h-FABP test assay and identifies fewer patients as being at very low risk and suitable for immediate discharge. In the modified MACS rule study, 18.9% of the patients were classified as very low risk compared to the original MACS 27% of patients classified as very low risk. This makes the modified MACS with automated h-FABP assay much more useful (Body *et al.*, 2015a). In 2016, the researchers then refined the MACS module with a version that required only a measurement by a high sensitivity troponin test (T-MACS). Therefore, the research suggests that MACS has greater sensitivity for ruling out ACS, however the assay required (h-FABP) is not widely used, minimising the utility of such a tool. T-MACS requires only the hs-cTnT used in most hospitals. It has a sensitivity of up to 98.7%, and it can rule out ACS in more patients than LoD and MACS-based strategies. The goal was to diagnose ACS with a single blood test at patient arrival at EDs in order to avoid the limitations of adding a new test (h-FABP) as mentioned earlier, which increases the barrier of implementing MACS in clinical practice. T-MACS demonstrated a sensitivity for a 30-day MACE of 98.7% from the original study and 96.3% from the external validation in Australia and New Zealand (Greenslade *et al.*, 2017). The rule could have enabled the 35.5% of patients, as the 'very low risk' group, to be rapidly discharged with zero (0.0%) missed AMI and one (0.4%) missed MACE. The MACS and T-MACS rules have been validated in two different studies with two different troponin assays (Body *et*

al., 2014c; Va Den Berg *et al.*, 2017). See Table 1.7 for a comparison of MACS and TMACS.

Table 1-5. Troponin-only Manchester Acute Coronary Syndrome decision rule

Present in Diagnostic algorithm	MACS	TMACS
Heart-type fatty acid binding protein (h-FABP)	Yes	No
High sensitivity cardiac troponin T (hs-cTnT)	Yes	Yes
ECG ischaemia	Yes	Yes
Sweating observed by treating clinician	Yes	Yes
Vomiting associated with presenting symptoms	Yes	Yes
Systolic BP <100 mmHg on arrival	Yes	Yes
Symptoms of Crescendo Angina	Yes	Yes
Pain radiating to the right arm/shoulder	Yes	Yes

(TMACS) and the Manchester Acute Coronary Syndrome decision rule (MACS) Variables

The T-MACS rule estimates the probability (p) of acute coronary syndromes as follows: $1 / (1 + \exp -(-4.77 + 1.71E + 0.85A + 0.61R + 1.42V + 2.06S + 1.21B + 0.089T))$ where E is evidence of acute ECG ischaemia, A is worsening angina, R is pain radiation to the right arm or shoulder, V is pain associated with vomiting, S is sweating observed, B is systolic blood pressure <100mm Hg and T is cardiac troponin concentration (ng/L) on arrival in the ED. For dichotomous variables, a value of '1' is entered for 'yes' and '0' for 'no'.

Table 1-6. MACS and T-MACS risk groups

Risk group defined by T-MACS	Probability threshold in the T-MACS Rule
Very low risk	<2%
Low risk	Between 2% and 4.9%
Moderate risk	Between 5% and 94.9%
High risk	>95%

Table 1-7. Comparison between MACS and T-MACS rule

	MACS	T-MACS
Number of patients included in the study	456 patients	703 patients
Cut-off used in the rule	Rise and/or fall of >9.2 ng/L (≥ 10 ng/L as levels was considered to be significant)	Lower Limit of detection of the high sensitivity Troponin assay
Percentage of very low risk group (rule out)	18.9%	40.5%
Percentage of high risk group (rule in)	11.1%	5%
The sensitivity of rule for 30 day MACE	Ranged from 100% -97.9%	98.7% from the original study and 96.3% from the external validation
NPV	97.7%	99.3%
30 day risk of ACS	<2%	<2%

1.5. Risk Stratification and Clinical Decision-making

When patients present ischemic symptoms, ACS diagnosis should be considered, but decisions regarding whether patients should be discharged or admitted with suspected MI should be based on biomarkers and ECGs combined with separating MI from other possible causes based on assessments by physicians (Smith *et al.*, 2015). Judgments of clinicians are critically important, as these also provide information for health care managers, scientists and other clinicians (Kienle and Kiene, 2011). Treatment plans and conclusions developed by clinicians are based on objective and subjective data, as well as radiologic results, laboratory data, physical symptoms and signs, and patient history. The diagnostic value of ED physicians' overall clinical assessments (gestalt) of ACS, in combination with ECGs and initial troponin results, has been evaluated in several studies (Chandra *et al.*, 2009; Kline and Stubblefield, 2014; Dezman *et al.*, 2017). One previous study has shown improved accuracy when combining clinical judgment with the ECG and biomarker findings in the assessment of 458 patients with chest pain in the ED. The researcher's strategy would have avoided admission for 23.1% (95% CI 19% to 28%) and 41.7% patients could have been discharged immediately (Body *et al.*, 2014a). Recently, a study evaluated the HEART score and clinical gestalt in chest pain patients

presenting in the ED and found a similar diagnostic accuracy for diagnosing ACS for both risk stratification tools (Visser *et al.*, 2014). Another study conducted in Sweden reported that patients with a non-ischemic ECG and normal initial troponin values shows that a history of UA chest pain can moderately predict UA, but a history of MI chest pain does not significantly predict MI. In addition, ACS is strongly predicted by an ischemic ECG and positive initial troponin values, and for ruling in ACS this appears to be more important than the pain history of patients. Finally, all ACS can be ruled out for patients younger than 40 years of age, when gestalt does not indicate ACS overall and is confirmed with patients' chest pain history. Therefore, in terms of ruling out ACS and ruling in ACS, this study argues that gestalt is better than its components (Mokhtari *et al.*, 2015).

Another study by Carlton *et al.* (Carlton *et al.*, 2015a) suggests that when no diagnostic ECG is used for assessing patients, gestalt could provide the most useful help for clinicians, and investigated 912 patients that had presented at ED with chest pain and were not given a diagnostic ECG. An ACS diagnosis was based on the rating given by physicians for the type of chest pain presented by patients, and to confirm their personal experience level where over two years of practice was shown to be experienced, and less than one year of experience was shown to be novice.

For both experienced and no-experience clinicians, the Receiver Operating Characteristic (AUROC) was 0.54 to 0.55 ($p < 0.05$), so there was a low correlation for the type of chest pain presented by patients with the final diagnosis of ACS, and when significant CAD on catheterisation was shown by examination of the patients and evaluated by clinicians, this did not change. For all comparisons, there was a low AUROC of 0.43–0.56, ($p > 0.05$) for experienced and novice physicians. These findings conclude that when compared with troponin and ECG testing, there is little diagnostic value shown by the judgment of physicians, so that there should be greater focus on rapid rule-out protocols and high-sensitivity assays in future research studies to enable patients to be discharged quickly from EDs when they are accurately identified as no-risk or low-risk patients.

1.6. Cardiac Biomarkers: Historical Perspective

The concept of the biomarker was defined in 1998 by the National Institute of Health Biomarkers Definitions Working Group as a contracted version of biological marker, which is an indicator that accurately measures and evaluates therapeutic intervention pharmacologic responses, as well as identifying pathogenic and normal processes (Strimbu and Tavel, 2010). However, patient outcomes and management provide the evaluation of whether a biomarker has a positive clinical value by its impact potential, so that before recommendations can be given for clinical use of new biomarkers, the American Heart Association in 2009 published detailed criteria that need to be met for newly developed biomarkers within a framework that is standardised (Hlatky and Hong, 2009).

There are significant medical challenges when approaches adopt biochemical diagnostic methods, as these are considered to be contentious and difficult (Rosalki *et al.*, 2004), because to achieve accurate diagnosis, specific criteria are needed for biomarkers used to diagnose myocardial ischemia. These criteria should include cost- effective, rapid and simple testing procedures, biomarkers must diagnose the extent of the injury by remaining within the blood for sufficient time, and at the time of ischemia, should be released rapidly into the blood, as well as being specific to cardiac muscles and demonstrating significant concentrations in the myocardium (Lippi *et al.*, 2006). Various cardiac biomarkers are shown as a timeline in Figure 1.5. During the 1960s, AMI was first diagnosed with a biomarker known as aspartate transaminase (AST), and during this decade the WHO incorporated this into its definition of AMI, but the characteristics of AST are not sufficiently specific for cardiac damage to be effective for detection, as it cannot specify cardiac muscle (Ladue *et al.*, 1954). AST returns to values that are normal five days after AMI, achieves maximum value in the blood from 15 hours and up to 28 hours, and after AMI, AST increases in the blood from 3 to 4 hours (Penttila *et al.*, 2000). Creatine kinase (CK) and lactate dehydrogenase (LDH) are additional cardiac biomarkers that began to be used during the 1970s and, although within the context of AMI, LDH is less specific for cardiac muscle than CK, both of these newly introduced biomarkers have no specific criteria for cardiac muscle. LDH

and the LDH-1 isoenzyme return to values that are normal after 12 days, achieve maximum value in the blood after 60 hours and until 144 hours, and after AMI these increase in the blood after 5 hours and until 10 hours (Christenson and Azzazy, 2006). CK returns to normal values after 72 hours, achieves a maximum value in the blood after 10 hours and until 20 hours, and after AMI increases in blood after 3 hours until 9 hours (Al-Hadi and Fox, 2009). In 1998, a RIA or radioimmunoassay was developed to detect myoglobin in serum, which is found in cardiac and skeletal muscle, and is defined as an oxygen-binding small cytoplasmic protein (Varki *et al.*, 1978). This became more useful than previous cardiac biomarkers, as it could be used for differential diagnosis of potential AMI, returned to normal values immediately, peaked between 4 and 12 hours, and after myocardial injury this is released into the serum after 1 hour, which is extremely early (Mair *et al.*, 1992; Keffer, 1997).

This innovative development replaced enzymatic assays that had been used previously, so this introduced protein concentration as an immunologic determination of CKMB mass, and in 1985, the first mass immunoassay for CKMB was reported (Chan *et al.*, 1985). The isoforms of the brain and the muscle are combined as letter symbols of B and M for forming the basis for MB, MM and BB isoenzymes, because the CK enzyme is present in humans. The highest concentration of the CKMB isoenzyme is shown in cardiac muscle, but for skeletal and heart diseases this can be very low or undetectable in the blood, whereas in the skeletal muscle it is ~1–3% total CK content, but in the myocardium it is 22% (Maisel and Jaffe, 2016). Within the first few hours of cardiac symptom diagnosis, CKMB sub-forms provide high accuracy, and diagnoses that are specific and reliable, which is reported in various studies (Puleo *et al.*, 1990; Puleo *et al.*, 1994). Rapid enzyme immunoassays was developed in 1990 to enable direct mass measurements of CKMB by using rapid enzyme immunoassays (Wu, 1999; Brandt *et al.*, 1990). For the purposes of this current study, the research focuses on heart fatty acid binding protein and troponin as cardiac biomarkers, which are discussed in detail in the following sections.

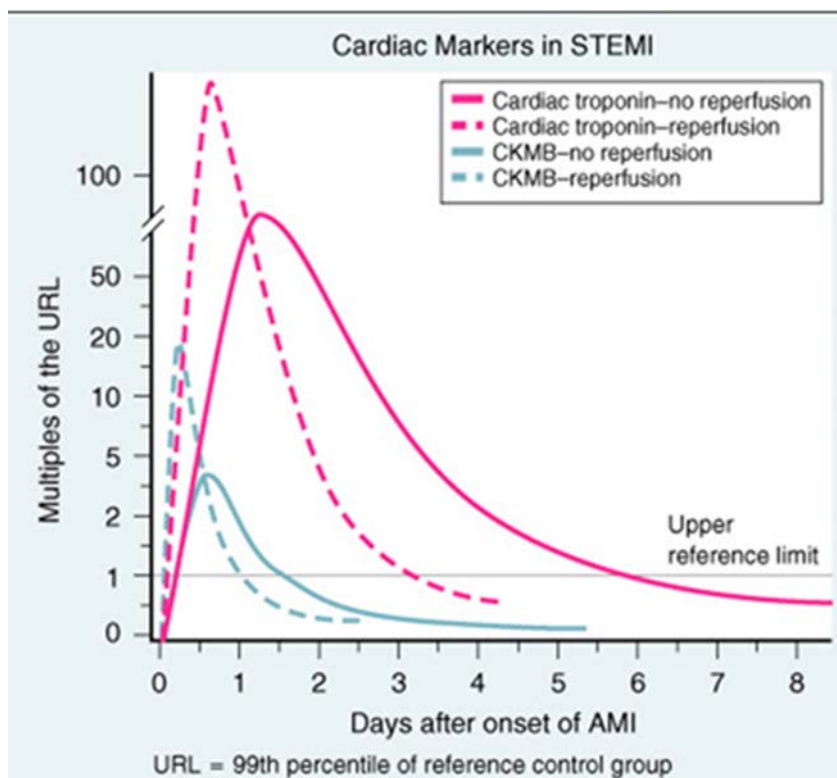


Figure 1-5. The kinetics of release of creatine kinase MB (CKMB) and cardiac troponin

Patients who do not undergo reperfusion are shown in the solid green and red curves as multiples of the upper reference limit (URL). Note that when patients with ST segment elevation myocardial infarction (STEMI) undergo reperfusion, as depicted in the dashed green and red curves, the cardiac biomarkers are detected sooner, rise to a higher peak value, but decline more rapidly, resulting in a smaller area under the curve and limitation of infarct size. AMI = acute myocardial infarction. Adapted from (Lilly and Braunwald, 2015).

1.7. AMI Definitions: Historical Perspective

The WHO published a definition of AMI in 1971 that applied the criteria of extensive laboratory testing, ECG or electrocardiogram abnormalities, and myocardial ischemia suggested by chest pain presented by patients, which changed its previous definition of AMI (Fox *et al.*, 2004). This definition was extended by the WHO in 1979 by including criteria of typical rise and fall patterns of LDH, CKMB and CK or AST activities (WHO, 1979). Between the late 1980s and early 1990s, myonecrosis cardiac troponin biomarkers were first developed, and were shown to be highly sensitive and specific, which contributed to MI being given its first universal definition and further updates (Alpert *et al.*, 2000). In 2000 and in 2007, further definitions of MI were

published that became universal, because new classifications of MI were included, as well as definitions that were more reproducible and standardised. The first of these definitions enabled the characterisation of the timing, trigger and size of AMI, and within the setting of AMI or myocardial ischemia, the classification of all degrees of myocardial necrosis (Alpert *et al.*, 2000). The second of these new definitions included a new classification with five categories, as well as updating the first definition of MI (Thygesen *et al.*, 2007). Subsequently, myocardial infarction's definition was developed further with a third universal definition because, within the settings of post revascularisation and critical illnesses, myocardial necrosis definitions were revised, and use of high-sensitivity assays to diagnose cardiac necrosis was developed significantly (Thygesen *et al.*, 2012a).

1.8. Cardiac Markers: Characteristics

Table 1.8 shows characteristics of cardiac markers that are optimal, as certain criteria are needed in secondary care or primary care when ACSs are indicated in chest pain reported by patients, so that myocardial injury can be evaluated by using an optimal plasma marker. Cardiac markers need to demonstrate low concentrations in non-cardiac tissue, but demonstrate high concentrations in the myocardium, and must provide cardiac specificity. As well as normal physiological conditions, it is also important to consider pathological states when evaluating the tissue distribution of potential markers. Therefore, CKMB or creatine kinase MB isoenzyme is not a perfect cardiac marker, because it is found in significant, but small, concentrations in skeletal muscle, and the absolute activity of the CKMB fraction in blood could be increased due to skeletal muscle injury (Babuín and Jaffe, 2005).

When evaluating injured myocardial tissue with a marker, the speed of its release in the blood is influenced by various factors, such as large molecules being released slower than small molecules, so that, when selecting a marker, its molecular size is important (Zaninotto *et al.*, 2004). The rate of release can also be limited by the intracellular location of the marker, so that structural proteins will appear later in the bloodstream than molecules located in the cytoplasm. See Table 1.9 for Acute Myocardial Infarction: Plasma Biomarkers Characteristics depends on the sensitivity of the assay have been used.

Table 1-8. Optimal Cardiac Marker: Characteristics

High sensitivity	Cardiac tissue shows abundance
High specificity	Non-myocardial tissue shows absence Non-diseased subjects cannot be detected in blood
Release	Early diagnosis due to rapid release Late diagnosis due to long half-life in blood
Analytical	Accurate Precise Short turnaround time Cost effective
Clinical	Patient outcomes improved as therapy is influenced Clinical studies provide validation

Table 1-9. Acute Myocardial Infarction: Plasma Biomarkers Characteristics

Marker protein	Molecular mass (kDa)	*First elevation in plasma after AMI (h)	Peak plasma concentrations	*Normalisation of plasma level (days)
h-FABP	14.5	1–2	6–12	1–1.5
Cardiac troponin I	22.5	3-8	12-24	7-10
Cardiac troponin T	37.0	3-8	12-24	7-10
Myoglobin	17.8	2-3	6-12	1-2
Creatine kinase MB	86	2-6	12-24	2-3

Abbreviations: AMI, acute myocardial infarction; h, hours; h-FABP, heart-type fatty acid-binding protein; kDa, kilo Dalton. *First elevation in plasma after AMI above the upper reference level of the marker protein. *Normalisation of plasma level (days) this level depends on time of reperfusion after arterial occlusion. Adapted with changes from (Willemsen et al., 2016).

1.9. Heart-type fatty acid-binding protein

In 1988 in a study of myocardial injury, h-FABP was first reported to have potential as a plasma marker (Glatz *et al.*, 1998). This plasma marker can supply substrates that are essential for energy production in the monocytes - ,in parenchymal cells, it functions as an intracellular fatty acid carrier - but also h-FABP is relatively small in size at 14.5 kDa (Glatz *et al.*, 2003). H-FABP is one of the most abundant cytosolic proteins, as it presents 1–2 % of total cardiac cytosolic proteins, and found in small quantities in parts of the brain, in distal tubule cells of the kidney and in slow twitch oxidative skeletal muscle (O'Donoghue *et al.*, 2006). In addition, when patients present with myocardial cellular damage, it is released in significant quantities to the interstitial space

and also rapidly, and then escapes into the vascular space through endothelial clefts due to the factors of small size, cardiac tissue abundance and cytosolic occurrence (VanNieuwenhoven *et al.*, 1996)

Troponin I is one and a half times larger than h-FABP at 22 kDa, and troponin T is three times larger than h-FABP at 37 kDa but, despite their smaller size, these and other troponins can present in plasma much later than h-FABP, because these need to be cleaved proteolytically from the contractile matrix after cellular damage. Therefore, to investigate possible cardiac injury, the first available plasma marker is h-FABP (Reddy *et al.*, 2016), and the cumulative release of h-FABP into the plasma enables estimations of infarct size, because the injured myocardium releases h-FABP when complete (Groot *et al.*, 1999). However, these estimations could be limited by renal clearance, as the kidneys are subsequently responsible for clearing h-FABP from the plasma (Al-Hadi and Fox, 2009). It is also affected by any coronary intervention that might be initiated immediately in a symptomatic patient (Wodzig *et al.*, 1998). H-FABP and other small proteins are cleared rapidly by the kidneys, which maintains a generally low plasma reference. Reports on plasma H-FABP reference values are limited (Niizeki *et al.*, 2007). The reported upper reference limit, i.e., the 99th percentile of the plasma H-FABP reference concentration, ranges from 5 ng/mL (Bathia *et al.*, 2009) to 9 ng/mL (Carless *et al.*, 2013) these levels are higher for men at 1.9 ng/ml than for women at 1.5 ng/ml (Glatz and Mohren 2013). Adults over the age of 50 years often present with decreased renal function and although circulating levels of h-FABP increase slightly with age. However, these effects should be taken into account in determining appropriate reference values for H-FABP.

1.10. Ruling out ACS: Combining Troponin and h-FABP

Numerous studies have investigated the utility of h-FABP for supporting the diagnosis of AMI and for prognostic stratification. A study by Liebetrau *et al.* (2014) has been reported that the h-FABP level is increased within only 15 min following trans coronary ablation of septal hypertrophy, a procedure mimicking AMI in patients with hypertrophic obstructive cardiomyopathy

(Liebetrau *et al.*, 2014). A recent meta-analysis published in 2017 by Xu *et al.* noted that routine use of h-FABP could not be necessary to diagnose AMI in early after-symptom onset even within 3 h (Xu *et al.*, 2017). Recent studies showed that h-FABP has high negative prognostic value for AMI in patients with suspected ACS, and it could be a supportive test when used in addition to high-sensitive troponin T. Recent clinical research has compared the diagnostic accuracy of h-FABP and high-sensitive troponin. A study by Kellens *et al.* (2016) evaluated the diagnostic accuracy of hs-TnT troponin and h-FABP tested by POC assays in 152 patients on admission, 6 h after admission and 15 hours after admission. The diagnostic sensitivity for ACS diagnosis when combining both tests was 82%, 88% and 89% respectively and the NPV for combining test was 70%, 82 % and 83% respectively, while the hs-TnT diagnostic sensitivity by its own was 72%, 93% and 94% respectively. These results showed that combining both hs-TnT and h-FABP tested by POC assays did not substantially improve diagnostic accuracy (Kellens *et al.*, 2016). Another more recent study analysed the data from 2,735 patients taken from eight earlier studies with qualitative h-FABP tests, and reported combining cTn with h-FABP increased pooled sensitivity 73 % to 91 %, but decreased pooled specificity from 94 % to 82% (Lippi *et al.*, 2013). Moreover, Keller *et al.* (2011), reported on combining quantitative hs-cTnI with h-FABP in a rule out strategy for ACS diagnosis and the NPV increased from 95.9 % to 97.6 %, PPV decreased from 66 % to 60 %, specificity decreased from 95% to 91%, and sensitivity increased from 73% to 85%. However, this finding indicated that the NPV of the combined test fulfils the diagnostic requirements for a rule-out strategy. In this study, the time between chest pain onset and admission to the ED was between 2 hours to 13 hours. However, it was not evaluated whether the performance of the combined test is dependent on the time of presentation of the patient (Keller *et al.*, 2011a). Similar findings were reported in another study, so that for MI diagnosis, when a conventional sTnI assay was combined with h-FABP this increased NPV from 92 % to 97 % and sensitivity from 77 % to 92 %, which shows significant enhancement. This study also reported that in patients who present to clinical settings within a few hours, combining hs-cTnI and h-FABP resulted in NPV and sensitivity achieving 100%, but overall

diagnostic accuracy did not improve when h-FABP was combined with hs-cTnI compared to hs-cTnI alone (Ruff *et al.*, 2013).

1.11. Cardiac Troponin

Cardiac troponin is the reference standard biomarker for the diagnosis of AMI. In the appropriate clinical context, the detection of a rise and/or fall of cardiac troponin is highly sensitive and specific for this diagnosis. However, troponin testing has two key limitations. First, as levels in serum or plasma can take several hours to rise, the diagnostic sensitivity of troponin testing is insufficient to allow ACS to be safely 'ruled out' and serial testing remains necessary. Second, because of the need to detect a rise and/or fall of troponin, serial testing is essential to differentiate chronic troponin elevations from those related to AMI. As a result, international guidance currently recommends serial testing over 6-12 hours. Troponin is not present in smooth muscle, but is a protein filament segment of skeletal muscles and the myocyte or contractile cardiac muscle cell (Zoltani, 2014). Figure 1.6 shows the troponin complex, tropomyosin, actin and myosin that form the contractile unit of the cardiac muscle cell. Actin produces the thin filament, and G actin units or globular proteins form a double coil, so that a tropomyosin protein covers each thin actin myofilament, and is linked to the troponin complex (Pinnell *et al.*, 2007). This is illustrated in Figure 1.7 TnT or Tropomyosin binding subunit, a TnI or inhibitory subunit, and a TnC or Ca²⁺ binding subunit form the heterotrimer protein known as the cardiac troponin complex. Calcium signalling regulates excitation-contraction coupling in cardiac muscle and skeletal muscle. Muscle contraction results from structural changes in the thin filament proteins initiated by calcium binding to TnC (Takeda *et al.*, 2003; Farah and Reinach, 1995). The TnI protein binds the heterotrimer to the thin filament closely when calcium is not bound by TnC. A hydrophobic section is exposed and the TnI prefers to bind to this, when conformational change is caused by the binding of calcium by TnC. This action results in the thin filament moving away from the complex, so that tropomyosin binds to TnT, and actin binding sites are then exposed that had been covered by TnI. All these actions contribute to muscle contraction. (Geeves and Lehrer, 2014; Tortora and Grabowski, 1996). cTnI or troponin I and cTnT or cardiac troponin T control calcium-mediated interactions between the myosin and actin, and are found in small quantities (5%) in the cytoplasm, but mostly in myofibrils (95%), as these are cardiac

regulatory proteins ('Troponins, Cardiac A2 - Wilson, David A', 2012). There is a unique structure for each of the three isoforms of cardiac muscle, fast-twitch skeletal muscle and slow-twitch skeletal muscle (Berridge *et al.*, 2013). However, after cardiac injury, specific genes code the regulatory proteins of the cardiac forms, so that these unique proteins are released into serum from the myocardium. Within skeletal muscle, some cardiac troponin T is present, but beyond the myocardium, cTnI is not present (Wu, 2017).

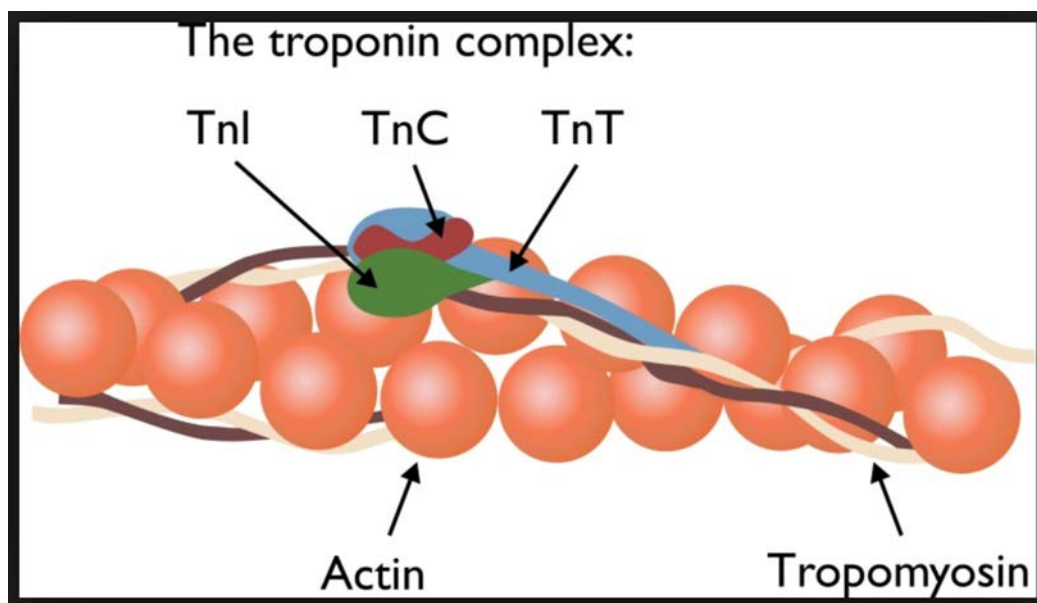


Figure 1-6. The troponin complex, tropomyosin, actin and myosin that form the contractile unit of the cardiac muscle cell

TnT or Troponin T, TnC or Troponin C and TnI or Troponin I. Adapted from (Hasić *et al.*, 2003).

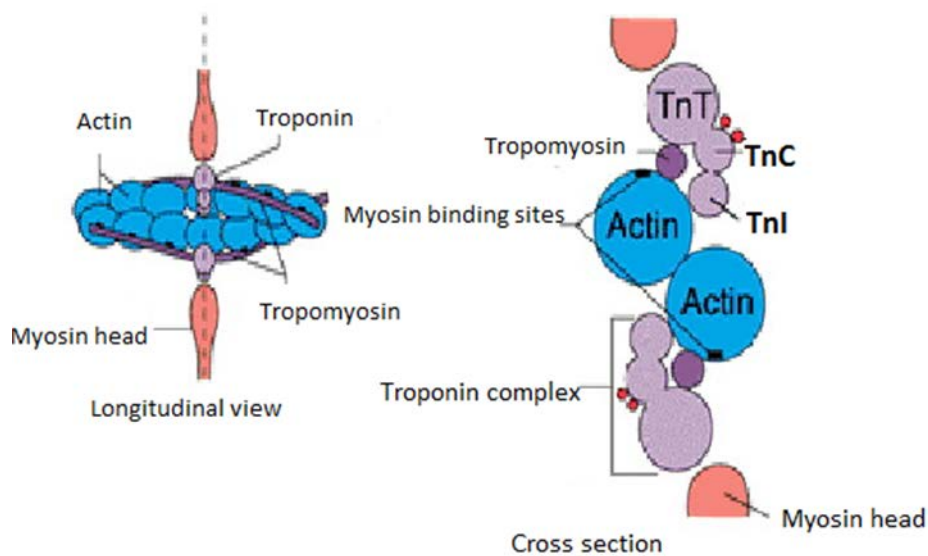


Figure 1-7. Troponin Complex Cross-Section

Actin produces the thin filament, and G actin units or globular proteins form a double coil, so that a tropomyosin protein covers each thin actin myofilament, and is linked to the troponin complex. Picture adapted from (Tortora and Grabowski, 1996).

Based on the literature review, cTnI and cTnT are known to be very sensitive and specific for myocardial damage despite the underlying cause of these two markers' elevation. Lately, it has been found that cTns can be elevated and indicate worse prognoses in many other conditions where ACS is not present. However, this finding affects the troponin validation as a cardiac marker for ACS without classic symptoms. Also, this finding makes the area of the troponin cut-off value challenging (Tanindi and Cemri, 2011; Al-Otaiby *et al.*, 2011). Table 1.10 illustrates the potential causes of troponin level elevation in blood, excluding AMI.

Table 1-10. Potential Causes of Troponin Level Elevation in Blood, Excluding AMI

<ul style="list-style-type: none"> -Sepsis -Pulmonary embolism -Hypothyroidism -Hypotension with arrhythmias -Hypertension and gestational -Haemoglobinopathy with transfusion haemosiderosis -Heart transplant -End of life stage renal failure -Over 30% of body affected by burns -Drug toxicity -Diabetic ketoacidosis -Patients who are critically ill -Congestive heart failure -Cardiotoxicity caused by cancer therapy -Carbon monoxide poisoning -Increased wall stress -Anaemia -Amyloidosis -Acute rheumatic fever
--

Table 1-11. Troponin characteristics

Characteristics	Troponin I	Troponin T	Troponin C
Weight	26.5 kDa	39 kDa	18kDa
Function	Actomyosin–ATP-inhibition subunit	Anchors troponin complex to the tropomyosin strand	Calcium binding subunit
Cardiac Specificity	Yes	Yes	No

1.12. Myocardial Injury: Time of Protein Release

Early diagnosis could be facilitated quickly following an injury, as some markers released into the blood make an early appearance. Following infarction events, there is a rise in cardiac troponins between four and six hours, which is similar to CKMB, and could take weeks for normal levels to return, but these peak between 12 and 24 hours. Previous research findings indicate that developing myocardial injury could be detected by using serial troponin measurements, because after an ischemic event, troponin elevation timing is important as, after myocardial injury, cTnI and cTnT are normally shown to be elevated between 4 and 9 hours, but also peak between 12 and 24 hours. Then, over the next 7 to 14 days, these enzymes remain in a state of elevation in the blood (Mythili and Malathi, 2015)

Therefore, emergency department clinicians need to know the time when chest pain was first presented in patients, because if the injury occurred within two hours of presentation at a hospital, commonly used assays might not be able to detect troponin elevations. However, this limitation could be overcome by observing patients that lack changes in ECG test results, because this could suggest a negative initial troponin, as well as suggesting ST segment myocardial infarctions. This period of patient observation could take several hours and involves cTn measurements, telemetry monitoring and physical examinations that are repeated. To enhance efficiency in EDs and ensure patient safety, studies have attempted to identify the shortest time needed for these observation periods, and findings report that after presentation, cTn evaluations should be repeated at 6 to 8 hour periods. However, there is pressure on clinicians working in EDs due to high numbers of patients presenting, so time points are not realistic options, and the only choices available are for clinicians to admit patients believed to be at health risk to observation units or inpatient wards in hospitals. One previous study suggests that for specific patients, repeat measurements could be taken at between two and three hours as a time point, based on findings related to troponin release and measurements (Collinson, 2015).

Findings also indicate that for patients with chest pain that developed from 6 to 24 hours before presentation at the ED, serial troponin measurements are

shown to improve sensitivities for ACS diagnosis (Nagurney *et al.*, 2005). In regard to risk stratification scores, researchers noted that in properly risk-stratified patients, chest pain evaluation with standard troponin I at patient presentation and two hours later lead to decreased disposition times without any significant increase in MACEs when compared to a conventional serial troponin measurement at time zero. After the observation period, the patient will follow the second troponin test in six to twelve hours. Those patients were classified as low risk on the “accelerated diagnostic protocol” in the study (Than *et al.*, 2014a). AMI diagnosis is often reached by choosing this marker within clinical settings, as it represents a significant rise and fall, and is recommended by the American College of Cardiology and European Society of Cardiology. The diagnostic criteria for MI enables clinical decision-making when healthy individuals are represented by a cut-off at the 99th percentile and normal, but above this indicates health concerns. Myocardial infarction was given its third universal definition in 2000 by a consensus group that was internationally based, and reported cardiac troponin or other cardiac biomarker values that are detected to rise or fall with a value higher than the 99th percentile and one of the following factors provides confirmation

- Autopsy or angiography that identifies intracoronary thrombus,
- Regional wall motion abnormality that is new, or imaging evidence of loss of viable myocardium that is new,
- ECG or electrocardiogram detecting pathological Q waves,
- Possible new left bundle branch block or possible new significant ST segment-T wave or ST-T, and
- Ischaemia symptoms.

1.13. Cardiac Troponin Assays

Serum cardiac markers of TnI were first classified in the 1970s, and of TnT were first classified in the 1980s, to indicate myocardial injury (Dean, 1998). The quantity of bound troponin is determined by a detection antibody, and cTn assays with either polyclonal antibodies or monoclonal antibodies to bind the molecule uses a two-sided sandwich immunoassay (Suetomi and Takahama, 1995). Cummins and colleagues reported the development of a cardiac-specific troponin I assay in 1987 for the first time (Cummins *et al.*, 1987), and

in 1989, Katus and colleagues reported the first cTnT assay (Katus *et al.*, 1989). To achieve a timescale that is clinically useful, results need to be achieved within a short period, so the assay should be quick, specific and sensitive, and the development of the assay has needed to address these factors (Hawkins, 2007). Proteolytic degradation, complexing with other molecules and phosphorylation are some of the factors that could affect cTn measurements, because there are various complexes and forms of cTn as it circulates in the blood, such as oxidised, reduced and phosphorylated cTn, T-I-C ternary complex, I-C binary and free cTn. However, manufacturers normally identify specificity for all antibodies, and the circulating form should be detected by antibodies (Sato *et al.*, 2012; Melanson *et al.*, 2007). Acute MI is diagnosed with a cut-off value above the 99th percentile with precision and a variance coefficient of around 10% within recommended professional guidelines, as discussed earlier, but the normal reference population of the 99th percentile has been insufficiently precise when using cTnI assays and cTnT assays, until recently (Thygesen *et al.*, 2012a). Conventional assays are known as initial first-generation troponin assays and are used to assess troponin for patients presenting at EDs with chest pain. Therefore, after the onset of chest pain between 6 hours and 12 hours, troponin can be detected in the blood with 100% sensitivity, and troponin is detected at microgram levels in these assays. Therefore, in guidelines to rule out MI, patients needed a second measurement assessment after between 6 and 12 hours (Thygesen *et al.*, 2012a). In healthy individuals, troponin concentrations are not accurately detected with conventional assays, and there are problems identifying upper reference limits, so that patients have needed to be categorised as undetectable or troponin-negative or detectable or troponin-positive following qualitative assessments (Ammann *et al.*, 2003). Conventional assays present a significant limitation, because after patients first present at emergency department, they often display a delayed rise in circulating concentrations of cTn. Therefore, new assays have been developed to overcome these weaknesses in analysis, and the high sensitivity troponin assay is capable of measuring troponin at picogram levels and nanogram levels, so that troponin can be detected in blood much quicker and with greater sensitivity (Apple *et al.*, 2012a).

An hs-cTn assay that would be ideal should measure below the 99th percentile for a healthy population at around 95% of normal values and achieve high sensitivity (Apple *et al.*, 2012a). A URL or upper reference limit applies accurate assessments of the 99th percentile with a coefficient of variation (CV) or confidence interval of 99%, and around 10% total CV for the 99th percentile for a population defined as healthy, and with variations of ethnicity, race and sex (Apple *et al.*, 2012a). Developing more sensitive assays should help to limit those patients in EDs whose myocardial injuries have not been previously detected, and many studies report that EDs that apply hs-cTn assays to evaluate patients considered to be at risk, achieve greater diagnostic sensitivity when compared with traditionally adopted assays when patients present at the clinical setting for the first time (Reichlin *et al.*, 2009). However, previous studies also highlight some limitations of using hs-cTn assays, such as inflated estimates of diagnostic sensitivity, because evidence suggests only 90% of diagnostic sensitivity at presentation with the cut-off set at the 99th percentile. Therefore, the LoD cut-off strategy has been applied by some researchers to improve evaluations.

Table 1-12. Potential Utility and Challenges of the High-Sensitivity Troponin Assay

Potential Utility	Potential Challenges
More rapid diagnosis in ACS	Not specific to etiology
Population screening	False positive interpretation
Prognostic information in stable patients	Biological variability
Drug development and cardiotoxicity	Lack of assay standardisation

Adapted from (Sherwood and Kristin Newby, 2014)

1.14. Point-of-care testing

Several approaches in sensor technologies and biomarkers have been developed recently. These allowed clinical diagnostic testing to be moved closer to the patient and to be tested outside the traditional lab environment (central laboratory) in the hospital. However, this has led to the development of devices called Point-of-Care Testing (POCT) (Quinn *et al.*, 2016). POCT is defined as diagnostic testing at, or near, the area of patient care. It is also known as bedside testing or near-patient testing (Luppa *et al.*, 2011). If

implemented appropriately, POCT can reduce the turnaround time (TAT) to therapeutic intervention, which has had an impact on improved patient outcomes, see Table 1.13 for several advantages of POCT. Before implementing POCT in the hospital, careful selection of the device should consider the local and international performance recommendations. Also, clinical needs assessment should be undertaken. (Clinical needs assessment defines unmet needs within healthcare). This approach, reached by gathering the information regarding the scope of POCT, also determines the potential impact of the current gap between traditional laboratory testing and clinician decision-making. To identify the clinical needs, several processes are used to analyse the available information regarding the clinical needs and to evaluate the analytical targets in addition to improving the assay performance and utility. This assessment could be approached by clinical research as this could determine the gaps and find answers on how to fill them (Weigl *et al.*, 2012). On the other hand, there is a range of barriers that might prevent successful implementation of POC testing that has been addressed in a recent literature review. See Table 1.14. for numbers of barriers to implementation of POC testing in hospitals

In the UK, the Oxford Diagnostic Horizon Scanning Programme was established in 2008 to present efficient information, which is needed for identifying and summarising the evidence for arising diagnostic technologies relevant to clinical settings. The available POCTs on the market are identified through systematic literature searches. The programme aims to interact with clinicians and the diagnostic industry to summarise the analytical, diagnostic accuracy from the manufacturer or previous researchers of the biomarkers tested by POCT. The finding then prioritised using a set of criteria. However, these criteria were divided into high priority or intermediate priority, see Table 1.15 for the recommended criteria for the prioritisation of POCT.

The detection of cardiac troponin is the most sensitive and specific biomarker of myocardial injury that is available to the physician in clinical settings and EDs (Wang *et al.*, 2017). The early identification or ruling out of the presence of ACS relies on a combination of signs and symptoms, cardiac biomarker

levels and findings in the ECG, according to the European Society of Cardiology (ESC) guidelines (Ibanez *et al.*, 2017).

Up-to-date, limited-need assessment studies on troponin testing by POCTs have identified diagnostic assessment gaps in hospital and emergency settings. In the area of developing a new algorithm in EDs to rule out AMI patients, or using a new platform assay for analytes in order to transfer a test from the laboratory setting to POC, it is essential to evaluate the diagnostic accuracy of the device, evaluate the agreement between POCT and central laboratory methods, and evaluate the POCT not only on comparative analytical significance, but also on its sensitivity and specificity in clinical practice. See Appendix 1.1

Table 1-13. Benefits of Point-of-Care Testing

The key benefits of point-of-care testing include:	
<ul style="list-style-type: none"> • Positive patient identification • Immediate diagnostic test results (reduced test and therapeutic turnaround time); • Reduction and/or elimination of specimen/sample transport; • Elimination of blood collection tubes and centrifugation with fresh whole blood specimen; • Reduced blood specimen volume; • Room temperature storage of test devices (few require refrigeration); • Data management and connectivity. • Connected POCT system benefits include: • Reduction in transcription errors; • Immediate data analysis—utilisation, QC, compliance, data mining; • Development of disease-specific algorithms—for example, tight glycaemic control. • New and novel approaches to patient care—for example, patient-centric care. 	

Table 1-14. Barriers to Implementation of Point-of-Care testing in hospitals

Barrier categories	Key issues
Economic issues	Cost per test of POC device is higher than central laboratory test Cost-effectiveness is difficult to compare between several sites Initial cost of implementation is high No specified budget for allocation Reimbursement structure within the hospital
Quality assurance and regulatory issues	Operation issues related to untrained or not competent staff Accreditation challenging to maintain regulatory compliance for a POC system Complex regulatory requirement associated with the accreditation Lack of investment in the research area of product qualification such as diagnostic studies and difficulty of the registration process of the product in the country
Device performance and data management issues	Lack of analytical performance studies in comparison to centralised laboratory testing
Connectivity and data management issues	Lack of acceptable connectivity capabilities Problems related to IT connectivity within the hospital
Management structure and clinical governance	Lack of interdepartmental management structure with clear clinical governance for POCT

Adapted with changes from (Quinn *et al.*, 2016)

Table 1-15. Recommended criteria for the prioritisation of POCT

High priority	Intermediate priority	Yes or No answer
Does the test have impact on morbidity and/or mortality of the disease?	Available information on the prevalence of the disease	
Reduces the number of people falsely diagnosed in target condition? Low false-negative result?	Is there a variation in treatment or patient outcomes resulting from POCT?	
Better diagnostic precision resulting in improvement in the delivery of diagnosis	The current diagnostic pathway for the disease could be improved by obtaining information in also less risky or in a way more acceptable to patients.	
Does the POCT improve the ability to rule out the disease?	Availability of safety profile of the POCT	
Is there available evidence of diagnostic accuracy in the setting needed to implement this POCT?	The impact of the POCT to rule in the disease	
Is the POCT would improve diagnostic efficiency or is it cost-effective?	It would be feasible to change current practice to incorporate this POCT	

Table data adapted with change from (Plüddemann *et al.*, 2010)

1.15. Accelerated chest pain protocols

Recent ESC guidelines were published in 2015, which supported the use of a new algorithm combining performing serial measurements of hs-cTn (at admission and repeated at 3 h) in addition to the clinical information available from chest pain patients in order to evaluate and risk stratifying patients according to their risk of AMI. In order to shorten the length of stay in the ED, the guidelines also suggested an algorithm that is proven for some, but not all, hs-cTn assays (including the hs-cTnT from Roche Diagnostics), and provides immediate or very early exclusion of AMI (Roffi *et al.*, 2015). This algorithm is based on either a “single sample strategy” (a single measurement at admission or at time 0) or two measurements of Hs-cTn performed at admission at time 0 and 1 h later. The threshold value for the “single sample strategy” is the limit of detection of the assay (LoD). Only 10% to 20% of patients who present to EDs with a suspected cardiac related chest pain are diagnosed with AMI (Six *et al.*, 2008). A high-sensitivity assay for cardiac troponin T (hs-cTnT) enables a more reliable detection of very low

concentrations of troponin. However, a single hs-cTnT measurement below the LoD or the limit of blank (LoB) may rule out AMI (Body *et al.*, 2012). Such tools have previously required patients to remain in hospital for long periods while undergoing further investigation as the turnaround time of the assay is about one hour. Moreover, because high-sensitivity assays are highly sensitive to detect troponin in blood, the increasing sensitivity of cTn assays lowers the number of potentially missed ACS diagnoses. This presents a diagnostic challenge because the gains in diagnostic sensitivity have inevitably come with a decrease in specificity and this results in ruling in more patients to the ED. On the other hand, the turnaround time of lab tests is about one hour. However, this requires the patient to be admitted to hospital and not being sent home, which leads to overcrowding in the ED. Finally, high-sensitivity assays are not specific for the etiology of cardiac cell death, and this results in increased responsibility for the clinician to interpret each test in a clinical context (Sherwood and Kristin Newby, 2014).

It is essential to establish the diagnosis of ACS rapidly in an ED to apply a suitable treatment for chest pain patients. An early rule-out of AMI prevents admission of patients without ACS and facilitates early discharge of patients. Moreover, a delay in rule-in of AMI may increase the risk of complications and mortality. In contrast, a delay in the rule-out of AMI may increase the time needed for patient assessments, unnecessary investigations, increased patient anxiety, as well as overcrowding in the ED. However, POCT allows measurement of biomarkers at short turnaround times (Larsson *et al.*, 2015). POCT for troponins should be implemented when a central laboratory has failed to provide test results within 60 min (Hawkins, 2007). Several members of healthcare teams, e.g. doctors, nurses, ambulance staff can run the POC device and read the result after adequate training. A recent rapid rule-out protocol (2 hours) using a POC biomarker test, TIMI risk score and ECG finding was shown to be safe in identifying a low-risk group (Than *et al.*, 2011a).

Aim and objectives

The aim of the current research is to evaluate several strategies that may enable clinicians to make accurate diagnoses based on information available

in the ED and a single blood test for cardiac markers at the POC. The study comprises the following objectives:

In chapter 3

The aim of this study was to evaluate the inter-observer reliability of a novel commercially available lateral flow immunoassay for h-FABP interpreted contemporaneously by staff in the ED. Both the predictive values and the kappa coefficient are supposed to depend on the prevalence and this should be noticed when results of different studies are compared.

In chapter 4

The aim of the current study was to evaluate the diagnostic accuracy of POC h-FABP lateral flow immunoassay (True Rapid, FABPulous BV) device for diagnosing or excluding AMI using a single test at the time of patient presentation to the ED and three hours later.

In chapter 5

The primary aim of this study was to determine the diagnostic accuracy of the Abbot i-Stat handheld POC assay. The study was also aimed to determine whether serial troponin testing using the Abbott i-Stat POC troponin device can accurately 'rule in' and/or 'rule out' the diagnosis of (AMI) within 3 hours of arrival in the ED, using either the conventional 99th percentile cut-off or a novel 'rule out' cut-off set at either (a) the limit of detection of the assay, or (b) the lower limit of the reportable range of the assay.

The objectives of this study were to address the following research questions: In patients with suspected ACS, can serial troponin testing within 3 hours of arrival in the ED using the Abbott i-Stat point of care device enable accurate identification of:

- a. Patients at sufficiently low risk of prevalent AMI, to enable safe avoidance of hospital admission.
- b. Patients at sufficiently high risk of prevalent AMI so that the diagnosis of an ACS should be considered 'ruled in'

In chapter 6

The aim of this study was to prospectively validate T-MACS with a contemporary POC cTn assay (i-Stat, Abbott Point of Care, New Jersey) in order to investigate the clinical diagnostic accuracy of i-Stat device to rule out AMI in EDs.

Chapter 2 : Materials and Methods

2.1. Methods

2.1.1. Detailed plan of investigation

2.1.1.1. Sitting

A multicentre, prospective diagnostic cohort study was undertaken in the Emergency Departments at 14 sites across the United Kingdom. During the study time frame of February 2015 to March 2017 there were 1,613 patients enrolled. Sites were invited to take part through being sent standardised, structured feasibility questionnaires (see Appendix 2.1). The information from the questionnaires was evaluated to ensure that the site had the capabilities and the resources to run the study. Interested sites were then selected. This would have the advantages of facilitating recruitment of greater numbers of patients and improving the probability that our results could be generalised to other patient populations. The details of each recruiting centre are shown in Appendix 2.2.

2.1.1.2. Current ED management process for patients with suspected cardiac chest pain

Patients who presented to emergency departments (EDs) at eligible sites with complaints of chest pain with symptoms suggestive of acute coronary syndrome were recruited prospectively into the study. At the time of the study, patients who came to the ED and reported chest pain were triaged by an emergency department nurse using the validated Manchester Triage System, see Figure 2.1. Comprehensive clinical, electrocardiographic and biochemical data were collected at the time of presentation using a custom-designed case report form (Appendix 2.3) for each patient who was eligible to participate in the study. In this observational study, patients were treated according to current departmental guidance for the management of cardiac chest pain, which is consistent with the guidance issued by the National Institute of Health and Care Excellence and the European Society of Cardiology (Alpert et al., 2000; NICE, 2010).

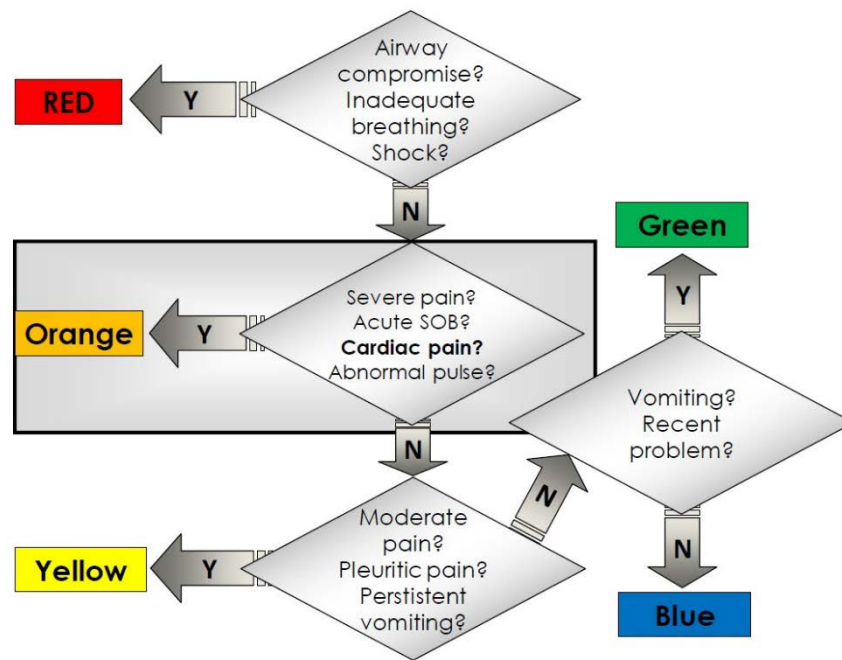


Figure 2-1. Chest pain pathway Initial assessment and treatment of suspected ACS in MRI hospital

Triage algorithm for patients with suspected ACS in MRI hospital

2.1.1.3. Selection of participant and recruitment process

2.1.1.3.1. Inclusion and exclusion criteria

Patients who presented to emergency departments at eligible sites with complaints of chest pain and symptoms suggestive of acute coronary syndrome requiring a hospital admission for further investigation were recruited prospectively. Patients were required to enrol within 12 hours of the onset of symptoms. All patients in the study were assessed and treated at the direction of the emergency physician.

Inclusion criteria: Adults patients (>18 years) who presented to the ED with pain, discomfort or pressure in the chest, epigastrium, neck, jaw, or upper limbs without an apparent non-cardiac source (compatible with the American Heart Association case definitions (26), which the treating physician believed warranted investigation for possible ACS; peak symptoms occurred <12 hours prior to presentation at the ED.

Exclusion criteria: Patients with unequivocal evidence of ST elevation myocardial infarction who were being immediately transferred for primary

percutaneous coronary intervention (PCI); Patients with another medical condition that would necessitate hospital admission; Patients who lacked the capacity to provide written informed consent.

2.1.2. Patient consent

Screening and consent patients were identified at the time of arrival by clinical staff and Clinical research nurses. Eligible participants were approached by investigators and given verbal and written information about the study. All patients underwent routine venepuncture for clinical purposes at the time of presentation to the ED. In order that clinical procedures including intravenous cannulation and routine blood tests were not delayed and because it was not feasible to obtain informed consent within minutes of arrival in the ED, research nurses drew initial blood samples prior to obtaining written informed consent. This allowed potential participants more time before providing full written informed consent (see Appendix 2.4 for the patient consent form).

2.1.3. Data collection

Each patient was assigned a study number at the time of presentation and each site engaged in the study had a unique number. At the time of inclusion, comprehensive clinical, electrocardiographic and biochemical data were collected and recorded in the custom designed Chest Pain Pathway designed for the patient's clinical record and the clinical report form (CRF) for the purposes of this study. The treating physician in the ED in accordance with the contemporary international standard filled this form for specific needed data which included details of the patient's complaint; previous clinical history; medication history; social history- including alcohol intake and tobacco use. Also, the family history of ischaemic heart disease found on physical examination; clinician's impression (including the clinician's judgement about whether or not AMI was present); 12-lead ECG findings (including the presence or absence of dynamic ECG changes such as T wave inversion or ST segment depression abnormal T inversion; dynamic change and left bundle branch block (LBBB) ; medication received pre-hospital and during the active study phase; disposition; findings of relevant laboratory tests and

medical history. The current location of patients who remained in hospital was recorded and any treatment given to the patient during their waiting in ED was recorded. All patients underwent a standard clinical assessment and received standard treatment within the ED as detailed above. Demographic details including the time of presentation to the ED, name, sex, age, date of birth, ethnic origin, address and telephone number, were also recorded.

2.1.4. Procedures for blood sampling and data recording

The troponin test by (i-Stat, Abbott Point of Care) and H-FABP by the FABPulous point of care device were undertaken immediately following venepuncture by the author at the MRI site or by a clinical research nurses in the other sites. All patients who had consented to providing blood for research purposes also had additional samples drawn at the same time they arrived at the ED for routine laboratory troponin investigations. Additional samples were stored in the SST II Advance, lithium heparin and EDTA vials and labelled with a unique study number. After processing, any samples that had been tested in 'real time' using point of care devices, the additional samples were centrifuged within 30 minutes of venepuncture at 2,500g for 10 minutes. Plasma and serum were separated and aliquots of 500 to 2,000µL were stored in vials labelled with the patient's unique study number. Sample characteristics (haemolysis, lipaemia, icterus) were recorded in the case report form. The serum and plasma were frozen at or -20°C or below within 4 hours of collection. Within 28 days, all samples were stored at -70°C or below for future analysis. The study protocol stated that each patient should have blood sent for reference standard laboratory-based troponin testing in accordance with contemporary national and international guidance. The sample-processing flowchart is illustrated in Figure 2.2. The overall schedule for blood sampling during this study is outlined in Table 2.1. The times of each blood draw, the result and the Sample characteristics (haemolysis, lipaemia, and icterus) were recorded on the case report form. (see Appendix 2.3). Disposition of patients (the patient's destination) after leaving the ED the time from arrival at the ED to troponin results was determined by review of hospital records this (included the upload time for the troponin results from the central laboratory).

Table 2-1. Sample collection schedule

Time point	Vials for collection (volume of whole blood)	Number of aliquots (volume) to be stored in the Biobank facility	Schedule for 'real time' analysis
Admission (T0) Samples collected as soon as possible after presentation and no more than 45 minutes after clinical blood samples	Lithium heparin x 1 (4ml) SST II Advance (BD Vacutainer) or similar (approximately 4.5ml) x 2 (total approximately 13ml; approximately 4.5ml taken as part of routine clinical care)	Serum: 2 (500µL) Lithium heparin plasma: 2 (500µL) Total 4	Lithium heparin: Troponin I (point -of -care, in the ED) Serum: High sensitivity troponin T (Roche Diagnostics Elecsys 2012) Whole blood (not to be stored): H-FABP (FABP-ulous)
3-hour (T3) Collected 3 hours (+/- 30 minutes) after T0 sample	Lithium heparin x 1 (4ml) SST II Advance (BD Vacutainer) or similar x 2 (total approximately 8.5ml)	Serum: 2 (500µL) Lithium heparin plasma: 2 (500µL) Total 4	Lithium heparin: Troponin I (point of care, in the ED) Whole blood (not to be stored): H-FABP

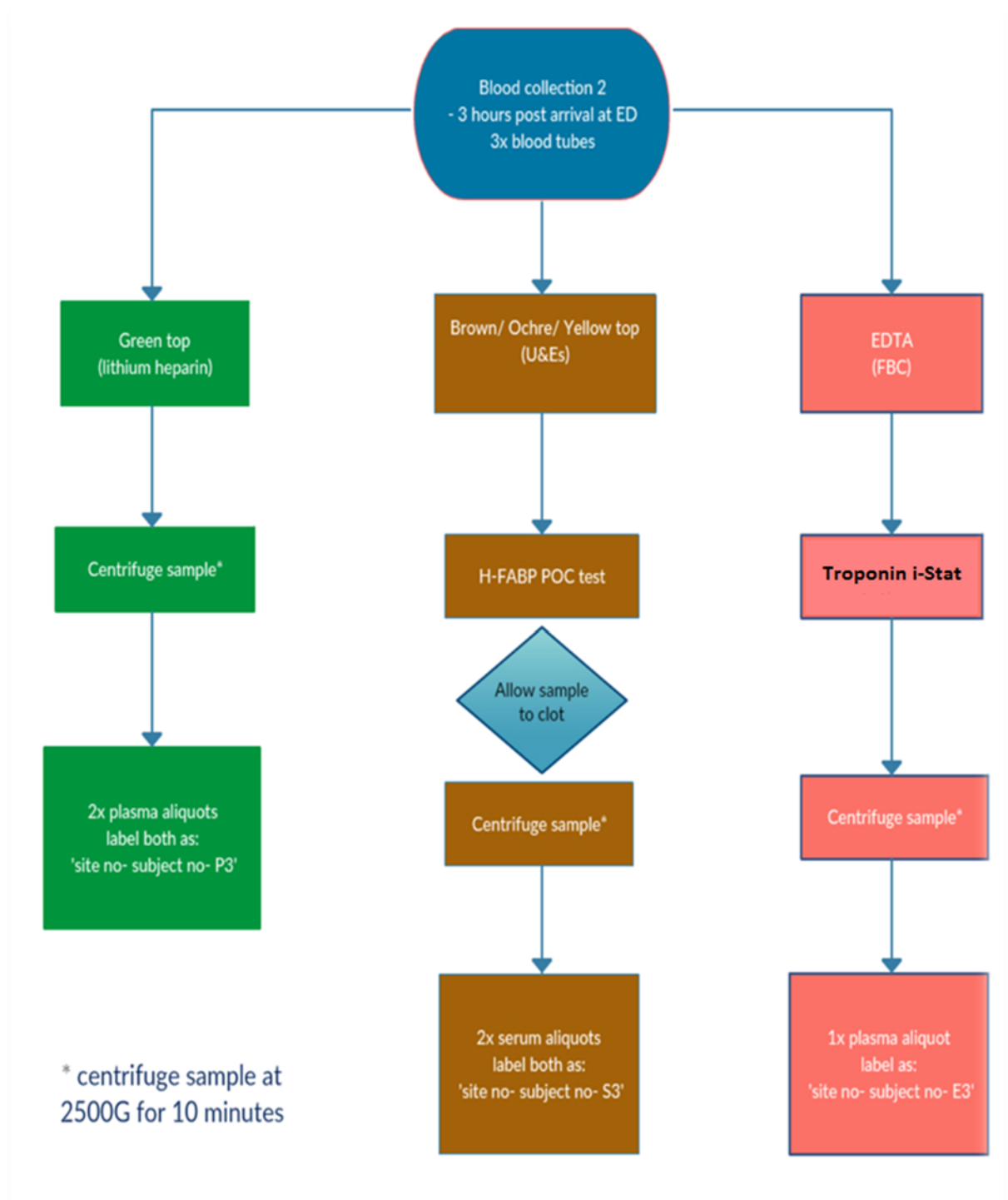


Figure 2-2. The sample-processing flowchart in the BEST study

2.1.5. Blood sample labelling and storage

After processing any samples that were tested in 'real time' using the i-Stat POC device and the FABPulous, the additional samples were centrifuged at 2,500g for 10 minutes. Plasma and serum was separated in aliquots of 500 to 2,000µL. Eight aliquots made up of 4 aliquots of serum and 4 aliquots of plasma (as described in Table 2.1) were stored initially at -20°C for less than 4 hours then the samples were transferred to -80°C and kept frozen for future analysis if needed.

2.1.6. Data recording

Each patient was assigned a special study number at the time of presentation. Each site participating in the study also had a unique number; special labels were designed for each patient in the study. Each site followed the same instructions and labelling system. Photocopies of the ED record, the cardiac chest pain pathway form (CRF), the initial and subsequent ECGs recorded in the ED and the consent form were all placed in a secure file for each patient. Follow-up data were also initially recorded manually using the custom designed forms for data collection. Completed follow-up forms were added to the patient's file. All patients records and CRF forms from each site were scanned and sent by email to the main site (MRI). All these records were reviewed and checked for any missing information and saved for the data analysis.

2.1.7. Ethical approval and Considerations

The National Research Ethics Service has ethically approved this study (Reference 14/NW/1344). Central Manchester University Hospitals NHS Foundation Trust and the and prince Sultan military medical city sponsored the study. This study was undertaken in compliance with the Declaration of Helsinki and local governance requirements. As per this study was conducted in the trust; a research passport has been obtained for the Author. Also, as this study was undertaken in the National Institute for Health Research (NIHR) funded time for the Chief Investigator and (NIHR) Portfolio adoption has been obtained (Reference UK CRN 18000). Patient information with a full

explanation of the study was given to all participating patients who potentially fulfilled the inclusion/exclusion criteria. All participants signed a study consent form before inclusion.

2.1.8. Follow up after 30 days

Patients were followed up 30 days after recruitment by the research nurses and patient were contacted by telephone, email, and letter or in person. All follow-up data and medical data were collected and recorded using custom designed forms (Appendix 2.3). These data covered all the medical records of the patient during these 30 days. If direct contact with a participant was not possible after 30 days, follow up information was obtained from their primary care practitioner. For all the living patients computerised hospital medical records were checked for details of patient admissions, outpatient appointment and relevant investigations. If the patients attended, any other hospital during the follow-up time the entire medical records from that hospital were also checked. All electronic medical records were checked and documented by the author/ research nurses. All patients were asked if they had any health problems or any adverse event, any serious adverse events over the last 30 days or whether they had experienced any cardiac event or acute myocardial infarction. Patients were also asked if they had undergone cardiac stress testing, coronary angiography or (thallium scan). All relevant information was recorded including details of blood transfusion or if they had undergone any dialysis for renal failure. Additionally, any haemorrhagic or bleeding complications were recorded. The date of patient discharge from hospital and total number of nights spent in an emergency department observation ward were also recorded. See Appendix 2.5. for patient follow-up form.

2.1.9. Tracking of missing data

For any missing results or data, the site was contacted via email and phone asking for the missing data. For any missing troponin result, frozen samples were checked and re-tested for the missing results by the author.

2.1.10. Outcome measures

2.1.10.1. Evaluation of interobserver reliability of h-FABP

In order to establish inter-observer reliability study in the clinical practice, we evaluated the inter-observer reliability of a novel, commercially available lateral flow immunoassay for h-FABP the FABPulous h-FABP True Rapid Test (FABPulous BV, Maastricht, Netherlands), which was interpreted contemporaneously by staff in the ED. To evaluate interobserver reliability, two independent investigators were asked to interpret the H-FABP result, blinded to each other's interpretation, for a subgroup of approximately 40 patients from the inclusion cohort in the study. A kappa score (κ) was calculated using SPSS version 19.0 or later (SPSS Inc, Chicago, Illinois). The sample size used in this primary analysis was calculated by consulting guidelines regarding sample size for reliability studies (Walter *et al.*, 1998) To demonstrate a κ of 0.8 with the minimal acceptable κ set at 0.6 and, setting the alpha (α) at 0.05 and beta (β) at 0.2, 39 patients were required to be assessed by two independent observers.

2.1.10.2. Acute myocardial infarction

Acute myocardial infarction (AMI) is defined according to the Third Universal Definition of Acute Myocardial Infarction as one that occurs within 30 days. Outcomes were adjudicated by two independent investigators (emergency physicians and consultants) in the EDs with reference to relevant clinical information but blinded to the results of the research investigators. The primary outcome was a diagnosis of AMI according to the Third Universal Definition of AMI. By asset of the inclusion criteria, all patients who experienced symptoms and signs consistent with myocardial ischaemia and developed a rise and/or fall of troponin to above the 99th percentile.

2.1.10.2.1. Primary analysis

For the primary analysis, the diagnostic accuracy of troponin I by (i-Stat, Abbott Point of Care) was evaluated at presentation, presentation plus 1 hour, presentation plus 2 hours, and presentation plus 3 hours. Sensitivity, specificity, positive and negative predictive values, and the area under the

receiver operating characteristic (ROC) curve were calculated. Statistical analysis was completed in SPSS version 19.0 or later (SPSS Inc, Chicago, Illinois) or MedCalc version 12.0 or later (Mariakerke, Belgium).

2.1.10.2.2. Secondary analysis

Validation of the Troponin only Manchester Acute Coronary Syndromes decision was made using a single POC troponin test in the ED.

2.1.11. Sample size/Power Calculations

The primary driver of sample size in a study of this nature is the number of patients with the primary outcome, which makes a conservative estimate of its prevalence desirable. Based on subtle differences in the inclusion criteria compared to similar studies run by previous study (Body, Burrows et al. 2015) The specificity of a troponin-based algorithm would be expected to be approximately 90% (Body *et al.*, 2011b) and the prevalence of the primary outcome in this cohort will be approximately 10% (Body *et al.*, 2010). Assuming that in the current study we identify an algorithm with 100% sensitivity and negative predictive value, the lower bound of the 95% confidence interval would be >90% for sensitivity and >99% for negative predictive value with a sample size of 605 participants. Accounting for potential loss to follow up and missing data (approximately 5%). The current study plan to include a total of approximately 650 participants.

2.1.12. Statistical techniques

- For the primary analysis, the diagnostic accuracy of point of care Troponin I testing was evaluated at presentation, presentation plus 1 hour, presentation plus 2 hours, and presentation plus 3 hours. The sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and, the area under the receiver operating characteristic (ROC) curve were analysed. All statistical analyses were performed using either SPSS version 22.0 (IBM Software, USA) or the MedCalc online calculator version.

- For the secondary analyses : T-MACS was computed using the original reported formula:

$1/(1+\exp(-(-4.77+1.71E+0.85A+0.61R+1.42V+2.06S+1.21B+0.089T)))$, where E is evidence of acute ECG ischaemia, A is worsening angina, R is pain radiation to the right arm or shoulder, V is pain associated with vomiting, S is sweating observed, B is systolic blood pressure <100mmHg and T is cardiac troponin concentration (ng/L) on arrival in the ED. For dichotomous variables, a value of '1' is entered for 'yes' and '0' for 'no'. However, POC cTn concentrations below the LoD (10ng/L) were entered as 9ng/L. We calculated sensitivity, specificity, positive and negative predictive values. (Details of the calculations will be described later in chapter 5).

2.2. Materials

2.2.1. Equipment and consumables

Table 2-2. Equipment and consumables

Item	Company/ code
BD Vacutainer Flashback blood collection needles	BD/ 368836
Microcentrifuge tubes, screw-cap with O-ring seal	Bunzl Healthcare/1153822
Pipette non-sterile	Bunzl Healthcare /13499108
Heparin used in BD Vacutainer	Bunzl Healthcare /vs367885
Serum separator tubes (BD SSTTM II Advance)	Bunzl Healthcare / VS367956
Centrifuge	
Cryo box with lid cardboard	Bunzl Healthcare /11872473
i-STAT handheld POC	Abbott Laboratories
i-STAT Troponin cartridge	Abbott Laboratories
H-FABP True Rapid Test®	(FABPulous BV,Maastricht, Netherlands)

2.2.2: i-STAT - Instrument Description and System Overview

2.2.2.1 Principle

The i-STAT cardiac Troponin I (cTnI) (i-Stat, Abbott Point of Care, 99th percentile 80 ng/L, LoD 10 ng/L), is a portable, hand-held POCT device capable of being used with a range of cartridges to measure a variety of biochemical parameters. This device is designed to be used at the patient's

bedside area and aimed to be used in critical care for the quantitative measurement of cardiac troponin I (cTnI) in whole blood. i-STAT test cartridges deliver lab-quality results at the patient site e.g. ED. The i-STAT (cTnI) test cartridge uses a two-site enzyme-linked immunosorbant assay (ELISA) method. Immunoassay is one of the most common protein assays which are becoming the gold-standard technique in clinical diagnostics for determining cardiac and other clinically relevant biomarkers. ELISA has been, since its discovery in the 1960s, a valuable tool in both clinical diagnostics and an important research area (Yolken, 1982). This assay has been adapted for point of care microfluidic based diagnostic assays to detect the protein markers in the blood by using of the binding interactions between antigens and antibodies. The first immunoassay for cTnI detecting was invented in 1987 by a radiation technique in an antibody–antigen–antibody sandwich format (Cummins *et al.*, 1987). The i-STAT cTnI test cartridge uses a two-site (ELISA) method (Abbott, 2015-2016). Antibodies specific for human cardiac troponin I (cTnI) are located on an electrochemical sensor made from a silicon chip. On the sensor silicon chip, there is also an antibody/alkaline phosphatase enzyme conjugate, which is responsible for a separate portion of the cTnI molecule. The whole blood or plasma sample is injected into the sensors enabling the enzyme conjugate to dissolve into the sample. However, the cTnI in the patient sample becomes labelled with alkaline phosphatase and is captured onto the surface of the electrochemical sensor during the incubation period, which is about seven minutes. The sample, as well as excess enzyme conjugate, is washed off the sensors. Inside the wash, fluid is a substrate for the alkaline phosphatase enzyme. The enzyme bound to the antibody/antigen/antibody sandwich separates the substrate releasing an electrochemically detectable product. The electrochemical sensor measures this enzyme that is proportional to the concentration of cTnI within the sample (Abbott, 2015-2016).

For the test analysis, approximately 16 μ L of whole blood was first introduced into the cartridge in the sample area. Second, the cartridge was closed and inserted into the i-STAT device. Finally, the result was viewed on the device screen within 10 minutes then the result was ready to be recorded in the case

report form. The procedure is described in Figure 2.3 with information from (Abbott, 2015-2016)

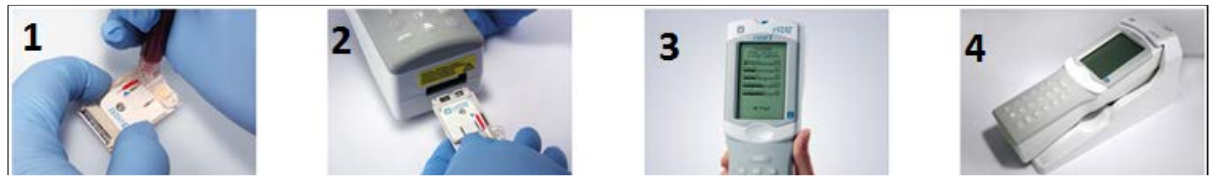


Figure 2-3. Four steps for the i-STAT troponin test. In the first picture, blood is placed in the i-STAT cartridge

Second picture, cartridge is inserted to i-STAT 1 Analyser. Third picture: after 10 minute result is showed in the analyser screen. Fourth picture: the i-STAT Analyser in the downloader/recharger (taken from Abbott, 2015-2016).

2.2.2.2 Cartridges

A whole blood sample of approximately 16µL is placed into the i-STAT cartridge sample area and then inserted to the device for analysis. The device automatically operates and controls the functions needed for the analysis, starting with the fluid movement within the i-STAT cartridge, calibration and then continuous quality monitoring. Troponin I cTnI cartridges were used once, and then discard. Cartridges are stored at room temperature 18 to 30°C or 64 to 86°F for 14 days

2.2.2.3 Analytical characteristics

Table 2.3 and 2.4 illustrating analytical characteristics of (i-Stat, Abbott Point of Care) and important analytical definitions in the use of troponin assays (Abbott, 2015-2016; Abbot, 2013).

Table 2-3. Clinical characteristics of (i-Stat, Abbott Point of Care)

Reportable range	Reference range (99th Percentile value)	Analytical sensitivity	Functional sensitivities (Limit of quantitation)
From 0.00 to 50.00 ng/mL (µg/L). Samples above the reportable range showed in the device screen > 50.00 ng/mL.	0.08 ng/mL	0.02 ng/mL.	The 20% and 10% functional sensitivities for the cTnI method are 0.07 ng/mL and 0.10 ng/mL, respectively

Note: Data for Table 2. Clinical characteristics of (i-Stat, Abbott Point of Care) from Abbott i-Stat troponin package insert information sheets (Abbot, 2013)

Table 2-4. Important analytical definitions in the use of troponin assays

Term	Definition
Analytical sensitivity	is the lowest cTnI level that can be distinguished from zero. The analytical sensitivity is defined as the concentration at two standard deviations from a sample at 0.00 ng/mL (Abbott, 2015-2016)
The limit of blank (LoB)	defined as the highest apparent concentration of a measure and expected when replicates of a blank sample containing no analyte are tested
The limit of detection (LoD)	is the lowest concentration of the measure and that can be detected at a specified level of confidence
The limit of quantitation (LoQ) Functional sensitivity	is defined, as the lowest concentration of measure and that can be determined with an acceptable level of precision. Which is defined as the cTnI level at which the test method displays a particular percent coefficient of variation (%CV)
99th Percentile value	Cut-off concentration used to discriminate myocardial necrosis. In laboratory medicine, the 99th percentile is typically determined from a normal, healthy population. This is usually the “positive/negative” cut-off used in clinical practice
Coefficient of variation (CV)	A measure of how consistently an assay is able to produce the same result on the same sample. A CV of 10% is the level of precision suggested for troponin assays

Note: Table terminology and definitions adapted with changes from (Armbruster and Pry, 2008)

2.2.3 The FABPulous B.V. (Maastricht, Netherlands) point of care device for Heart Fatty Acid Binding Protein detection

The concept of this point of care (POC) test is using a combination of rapid whole blood filtration and subsequent lateral flow immunoassay in order to

deliver an accurate result within 5 minutes. The device is disposable, simple to use and needs no equipment. However, by design, (POC) devices are referred to as near-patient, to rapidly deliver bedside test results in order to support better patient management. This allows the FABPulous for H-FABP test to be completed by non-biomedical staff such as nurses and doctors. Also the short turnaround time of the test makes it useful for assessment of the patient's blood in a busy environment such as an ED which needs to deliver a quick result at the patient site.

2.2.3.1 Principle

This is a qualitative, lateral flow immunoassay (LIA) combined with an integrated, one-step plasma filtration device. Inside the cartridge, a strip is present which contains an antibody that specifically binds the H-FABP that is present in the patient's whole blood sample. The device consists of two parts: first, a blood collector to absorb the blood and a second small part that is docked onto the blood collector. This design allows a pressure-controlled filtration to produce diluted blood plasma (Figure 2.4). The cartridge contains a filter stack in addition to the (LIA) strip. The blood collector is placed in a padded area in the cartridge, after which the buffer contained within the blood collector is pressed in a controlled manner through the advanced filter stack to produce a diluted blood plasma, which then is guided to a lateral flow strip. The (LIA) takes place in the second part of the device. The stripe contains a control line (C) that detects free conjugated mouse H-FABP antibodies that shows the effective passage of buffer through the device. The test line (T) detects any H-FABP from a patient sample that is sandwiched by the colloidal gold-labelled mouse anti-H-FABP conjugate. see Figure 2.4.

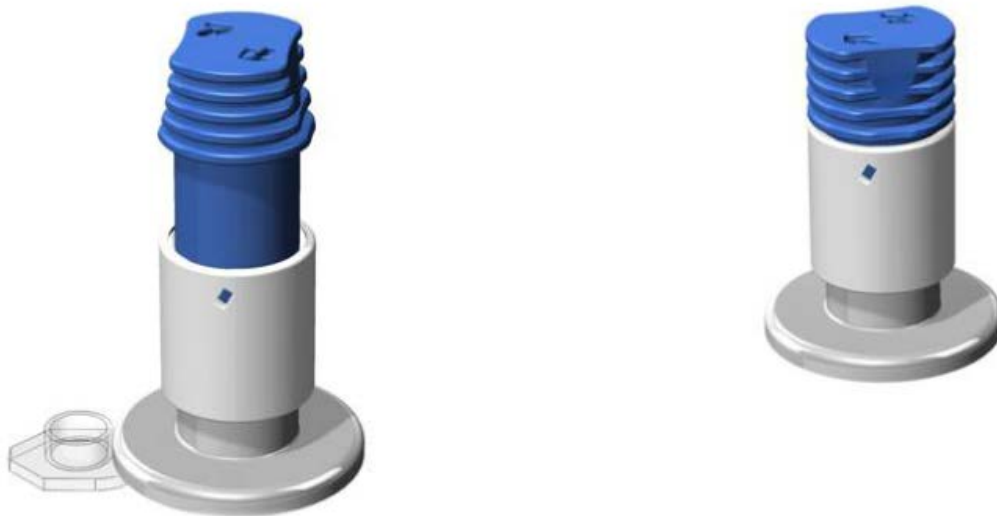


Figure 2-4. Plasma Separation Device.

Picture taken from (Fabpulous, 2016)

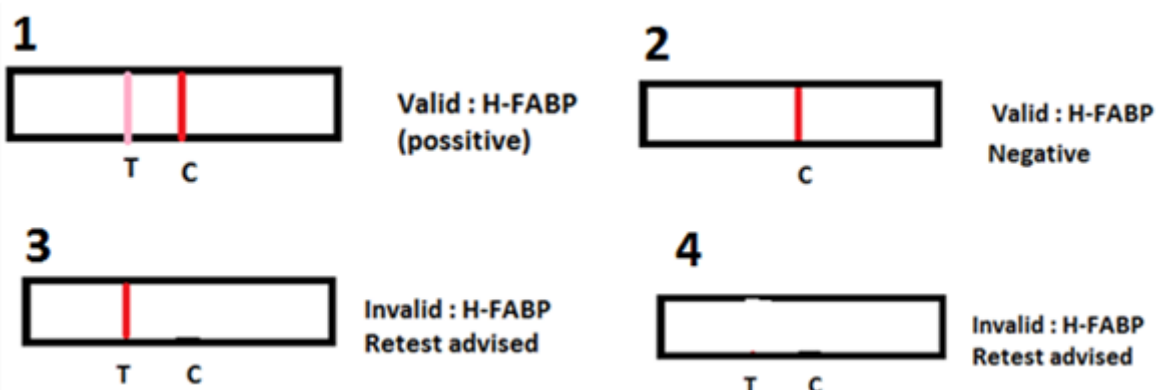
2.2.3.2 Sample type

Whole blood only.

2.2.3.3 Interpretation of test results

H-FABP is present in concentrations above 4ng/mL. The appearance of a line at the position marked with a “C” is the control line, indicating that the test has performed correctly. However, the test result is only valid when this control line is visible. A positive result is the appearance of a coloured line on the stripe if the concentration of H-FABP is elevated. This method takes just five minutes see Figure 2.5 for the interpretation and Figure 2.6 for a positive result in the FABPulous device (Fabpulous, 2016).

Figure 2-5. Result interpretation in The FABPulous device



Picture 1, valid test with the appearance of the positive result in both control and H-FABP tests are visible. Picture 2: valid test showing negative result for h-FABP with no visible line and control is visible. In picture 3: invalid test showing positive result with visible line in test line but no visible line in the control line. Picture 4: showed invalid H-FABP test with no visible line for both control and H-FABP test. In this cases test 3 and 4 tests should be repeated. Picture modified with permeation from (Fabpulous, 2016).



Figure 2-6. Positive result in the FABPulous device.

H-FABP test positive result with a visible line for both control and H-FABP test area. Picture taken from (Fabpulous, 2016)

2.2.3.4 Analytical characteristics

The diagnostic sensitivity of the test is 93.6% and the specificity 67.4% from three hours to eight hours after onset of symptoms. The cut-off is 4ng/ml at

which 95% of H-FABP concentration is detected note: information taken from insert sheet of The FABPulous device (Fabpulous, 2016).

2.2.4 High sensitivity troponin assay and contemporary troponin assays

The reference standard for AMI was serial central laboratory cTn testing over at least 3 hours (for high sensitivity assays) or at least 6 hours (contemporary assays). At the time of writing this thesis and pending further evidence and international guidance, a significant rise and/or fall of troponin was considered to be >9.2ng/L for high sensitivity troponin T (Mueller *et al.*, 2012) and at least 20% for all other assays.

**Chapter 3 : The inter-observer reliability of a novel qualitative point of
care assay for heart-type fatty acid binding protein**

Abstract

Background: Heart-type fatty acid-binding protein (h-FABP) may help to improve the early diagnosis of acute coronary syndromes in patients presenting to the Emergency Department (ED) with chest pain. A novel qualitative point of care h-FABP lateral flow immunoassay (True Rapid, FABPulous BV) could provide results to clinicians within just 5 minutes. Given the qualitative nature of this test and prior to evaluation in a large diagnostic study, we aimed to determine inter-observer reliability when interpreted contemporaneously by staff in the ED.

Methods: In a nested prospective cohort study including adult patients with suspected cardiac chest pain, venous blood samples were tested for h-FABP (FABPulous BV) on arrival and 3 hours later. Each test result was independently interpreted by two different investigators after 5 minutes. The investigators were blinded to each other's interpretation and recorded their findings on separate case report forms. We determined interobserver reliability by calculating the Cohen's kappa score and 95% confidence intervals.

Results: A total of 43 test results (from 31 patients) were each interpreted by two independent investigators. Absolute agreement between investigators was 93.0%, with a Cohen's kappa score of 0.81 (95% CI 0.6 – 1.0), indicating near perfect agreement. In total, there were three (7.0%) disagreements. In each case one investigator reported a 'weak positive' result while the other interpreted the result as 'negative'.

Conclusions: These findings demonstrate the interobserver reliability of a qualitative point- of-care h-FABP assay. Further work must evaluate diagnostic accuracy and determine the clinical implications of the small rate of disagreement.

3.1. Introduction

In patients presenting with acute chest pain, early and accurate diagnosis of acute myocardial infarction (AMI) permits interventions to reduce the mortality rate (Milosevic *et al.*, 2016). It is important to identify patients who are not suffering from AMI, who can be sent home safely. Heart-type fatty acid binding protein (h-FABP) has been suggested as a cardiac marker to reliably detect myocardial ischemia in the absence of necrosis for initial identification and for differentiating patients with chest pain of symptoms other than coronary ischemia. It could also provide clinical utility complementary to that of cardiac troponins (cTn) the gold standard in acute myocardial infarction (Collinson *et al.*, 2017). While levels of cardiac troponin may take several hours to rise after the onset of AMI, (h-FABP) is released faster after myocardial injury because of its lower molecular weight and cytoplasmic location see Figure 3.1 (Banu *et al.*, 2014). When the heart muscle (myocardium) is damaged, h-FABP is immediately released into the bloodstream prompting rapid elevation in the concentration of h-FABP (Willemsen *et al.*, 2015a). Its plasma concentration increases at least 30 min following the onset of AMI and return to the normal range within 12–24 hour in patients without renal impairment diagnosis (Glatz and Renneberg, 2014). There has been much interest in h-FABP as a biochemical marker. Several studies have shown to improve the patient's diagnosis as an early diagnostic marker in ACS (Collinson *et al.*, 2017; Carroll *et al.*, 2013). Among patients presenting at the (ED) with suspected ACS, h-FABP has been shown to be a strong independent predictor of major adverse cardiac events (MACE) within 30 days even after accounting for high sensitivity cardiac troponin, electrocardiographic (ECG) changes and clinical findings. As such, h-FABP was incorporated in the Manchester Acute Coronary Syndromes (MACS) decision rule (Body *et al.*, 2014). This rule effectively risk stratifies patients following a single blood test for high-sensitivity cardiac troponin and h-FABP, taken at the time of arrival in the ED. It can be used to identify subjects who could have ACS immediately 'ruled out' (thus avoiding unnecessary hospital admission) and others in whom the diagnosis could be immediately 'ruled in'. The MACS rule has now been validated with an automated laboratory-based h-FABP assay (Body *et al.*, 2014c). However,

the requirement to run the h-FABP assay may present a barrier to clinical implementation, as this assay is not routinely used for other indications.

Several point-of-care tests (POCT) designed to detect h-FABP in whole blood samples are available and could reduce the time taken for the result to be available compared to laboratory blood tests, thus allowing better treatment and outcome (Mad *et al.*, 2007b). The availability of a point of care (POC) assay may therefore support the diagnosis of chest pain patient in ED and this could minimise the barrier of the h-FABP test to be implemented in clinical decision rule.

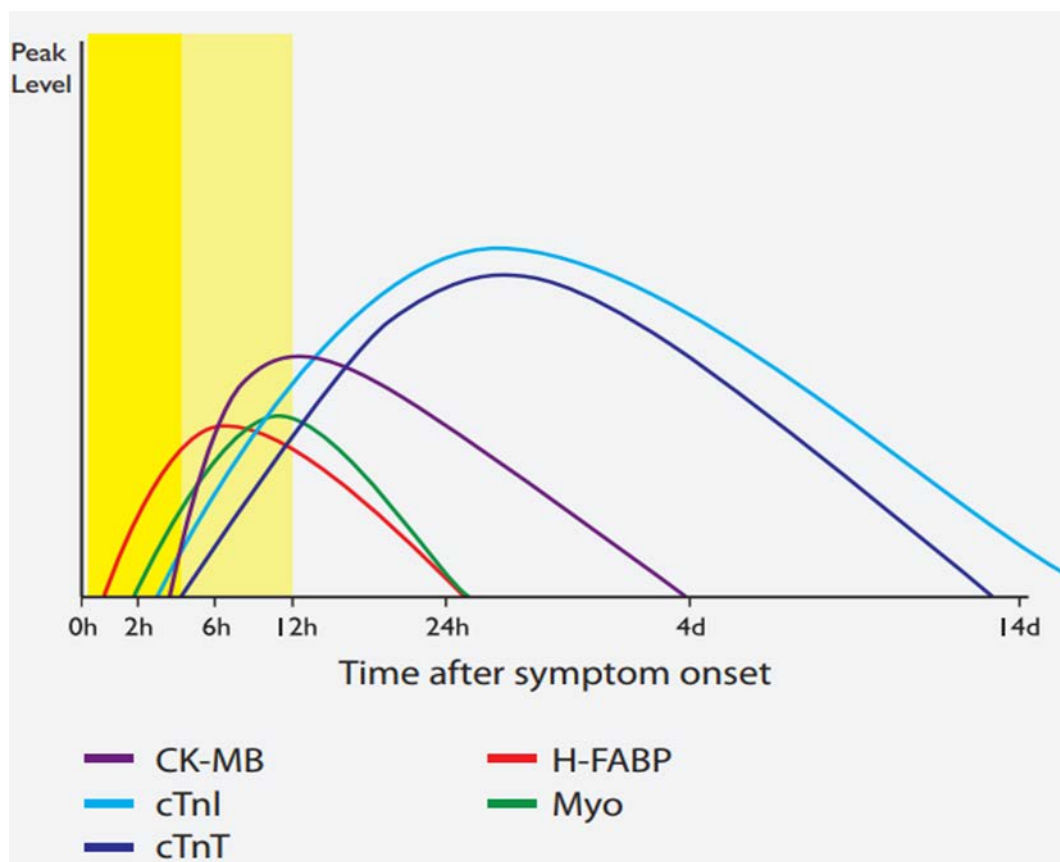


Figure 3-1. Release of h-FABP and cardiac troponins from the injured heart into plasma after acute myocardial infarction (AMI)

Clinical biomarker measurements are usually subject to various types of error. This error due to imperfect lab conditions (e.g., operator error, contamination or variable storage conditions) this lead to having a measured valued differently from the correct value. However, assessing the quality of a biomarker is important, and this process consists of determining if the

biomarker has sufficient reliability and acceptable validity (Bartlett and Frost, 2008). Reliability refers to “the degree to which the results obtained by a measurement procedure can be replicated” (Faerstein, 2016). The reliability in statistics is most often described in terms of interrater and intrarater reliability. Interobserver agreement refers to the agreement between the determinations made by two different observers (Looney, 2002). However, In order for any biomarker test to be used properly, it is essential that several parameters be established regarding the test before applying the test in clinical practice. Also, sufficient monitoring requires ensuring that the device meets the quality demands of psychometric properties such as feasibility in clinical practice, validity, reproducibility, sensitivity, specificity, predictive values, and finally the likelihood ratios. The most basic parameters that are essential to be established regarding any clinical biomarker are that it demonstrates a sufficient degree of reliability and validity between users (Kha and Chien, 2001). Interestingly, if these two important parameters are not met, then the test’s value in assisting clinicians to decide on the patient diagnosis from the patient management plan or monitor a patient’s improvement is questionable (Bruno, 2011). Since the essential purpose of the diagnostic test is to support the decision making in determining whether a patient has or does not have a certain condition (Deeks and Altman, 2004). Reliable evidence from clinical trials is needed for making appropriate decision-making. However, in the context of clinical trials, the quality of a diagnostic study is evaluating the accuracy of the diagnostic test. In ED, It is essential to choose TAT goals that lead to improved patient care and clinician efficiency in addition to improved satisfaction for both patients and clinicians. POCT has an overall advantage in ensuring timeliness of test results. However; the accuracy and reliability of the results are often in question and must be addressed as well as meets the analytical goals. POCT devices must be sufficiently accurate and precise to achieve the clinical purposes for which they are designed. Assessing the reliability of the device is essential since the performance of a diagnostic test is reliant on the ability of the observer to reliably and predictably evaluating the presence or absence of a positive result.

There is various commercially available qualitative and quantitative POC h-FABP assays in the market see Figure 3.2. Quantitative assays report results as continuous variables and thus provide clinicians with a 'number'. The disadvantages of quantitative assays include the financial cost of analysers and the requirement for regular quality control and calibration, which is often delegated to already over-stretch clinical staff. Qualitative assays, which provide a simple dichotomous result (positive or negative) have the advantage of removing the need for a separate analyser, thus reducing financial cost and saving staff time, although these potential benefits may depend on institution-specific factors. Qualitative assays do, however, require interpretation, which has an element of subjectivity. In this study, The FABPulous h-FABP results of a lateral flow immunoassay were performed by determining the existence or absence of a test line next to a control line. Because lateral flow immunoassays necessitate interpretation, which has an element of subjectivity, an important first step in their evaluation is to establish inter-observer reliability in clinical practice. The performance of a diagnostic test is dependent on the accuracy and the ability of the observers to reliably and predictably assess the presence or absence of a positive result. The reliability of the test must in some cases be measured by a determination of reproducibility. The reproducibility is measured by comparing results of repeated examinations of the same patient. A Kappa coefficient test is used to measure the reproducibility, which adjusts the observed agreement for an expected chance agreement.



Figure 3-2. A variety of POCT for heart fatty acid binding protein testing

This picture illustrates different types of POC for h-FABP testing, **(1)** is the qualitative the FABPulous h FABP testing, **(2)** qualitative cardio-check POC for h-FABP. **(3)** Colloidal Gold-labelled Quantitative Immunoassay Analyser for h-FABP testing

3.1.1. Aim and objectives

The aim of this study is to evaluate the inter-observer reliability of a novel commercially available lateral flow immunoassay for h-FABP interpreted contemporaneously by staff in the ED. Both the predictive values and the kappa coefficient are supposed to depend on the prevalence and this should be noticed when results of different studies are compared.

3.2. Method

3.2.1. Data collection and processing

In a prospective cohort study nested within the larger Bedside Evaluation of Sensitive Troponin (BEST) study, the main thesis included consenting patients who presented to the ED with suspected cardiac chest-pain within 12 h of symptom onset. Participants were selected for inclusion in this sub-study by

accessibility sampling, dictated by the availability of two trained independent observers. Venous blood was drawn on arrival and 3 h later (without additive). Fresh whole blood samples were then immediately tested for h-FABP using the FABPulous h-FABP True Rapid Test (FABPulous BV, Maastricht, Netherlands; calibrated to provide a positive result at 4 ng/ml) according to manufacturer's instructions. This is a lateral flow immunoassay (LIA) combined with an integrated, one-step plasma filtration device that delivers whole blood into a correctly and consistently diluted plasma (Glatz and Renneberg, 2014). After 5 min, two independent observers including clinical research nurses, emergency physicians and my self-interpreted each test. It should be pointed out that the staff who interpreted tests in this study had the same background as the routine clinical staff who would be expected to interpret the h-FABP point of care test results in routine clinical practice. Moreover, each investigator had received a two-hour training session on how to perform and interpret the test results.

The observers were both blinded to each other's interpretation and recorded their interpretation on separate case report forms. The investigators interpreted the result from the same test immediately. Interpretation was timed using a stop clock to ensure protocol adherence and a maximum delay of 2 min was permitted between the two interpretations. The investigators interpreted the result from the same test without delay. Interpretation was timed using a stop clock to ensure protocol adherence and a maximum delay of 2 min was permitted between the two interpretations.

3.2.3. Statistical methods

The most basic approach to assess interrater agreement is to calculate the observed proportion of cases where the observers agreed. This approach alone is clearly insufficient, since it does not consider the fact that a certain amount of the agreement would be expected purely by chance. The kappa statistic is a useful method of assessing the extent of agreement beyond that expected by chance. This method is appropriate for assessment of interobserver reliability with regard to binary or ordinal scale data (Fleiss *et al.*, 2013).

The kappa coefficient was calculated after cross-tabulating the results from each interpreter in a 2x2 table using SPSS version 20.0 (SPSS Inc, Illinois).

Kappa (κ) can be calculated by determining the proportion of agreement that could have been achieved beyond that expected by chance alone, as follows: (Viera and Garrett, 2005).

$$k = (PO - PE) / (1 - PE) \text{Kappa}$$

Where PO is the proportion observed and PE is the proportion expected by chance alone. Kappa is scored from 0 to 1. A κ score of 0 indicates that there is no agreement beyond that expected by chance, whereas a score of 1 indicates perfect agreement. The (κ) score was further categorised according to Landis and Koch (Landis and Koch, 1977) which is shown in **Table 3A.1**. To demonstrate a κ of 0.8 with the minimal acceptable κ set at 0.6 and, setting the alpha at 0.05 and beta at 0.2, would require 39 patients to be assessed by two independent observers.

3.2.4 Sample size calculation

Sample size was calculated according to guidelines for reliability studies (Walter *et al.*, 1998). However, in order to demonstrate a κ of 0.8 with the minimal acceptable κ (lower boundary of the 95% confidence interval) set at 0.6 and, setting the alpha at 0.05 and beta at 0.2, we would require 39 patients to be assessed by two independent observers.

Table 3-1. The interpretation of Cohen's kappa classification

The interpretation of Cohen's kappa classification by Landis and Koch is often used to judge the strength of agreement for κ (Landis and Koch, 1977)

Value of kappa	Level of agreement
<0	Poor agreement
0.0–0.2	Slight agreement
0.2–0.4	Fair agreement
0.4–0.6	Moderate agreement
0.6–0.8	Substantial agreement
0.8–1.0	Almost perfect agreement

3.3. Results

3.3.1. Baseline characteristics

The study was conducted from 2nd April to 8th May 2016. A total of 43 test results were interpreted by each of two independent investigators for this study (26 drawn on arrival and 17 at 3 h). The tests were drawn from a total of 31 patients (12 of whom had tests from both arrival and 3 h later interpreted by two investigators). The mean patient age was 60.6 ± 12.49 years with 15 males (48.4%) and 16 females (51.6%). Fourteen patients (45.2%) had hypertension, six patients (19.44%) were had hyperlipidaemia, and two patients had diabetes mellitus one patients (3.2%) had type 2 and one patient (3.2%) had type 1 diabetes. Five patients (16.1%) were current smokers, one patient (3.2%) had renal impairment, 10 patients (32.3%) had a history of myocardial infarction and 8 patients (25.8%) had a history of prior angina and 7 patients (22.6%) went to percutaneous coronary intervention (PCI). Admission ECGs were normal in 27 patients (87.1%), four patients (12.9 %) had acute ischaemia, eight patients (25.8%) had significant ST-segment depression and tow patients (6.5%) had ST-segment elevation. (Table 3A.2).

3.3.2. Result interpretation

A total of ten observers interpreted h-FABP results, including four doctors (emergency physicians), five nurses and one medical technologist. A total of 21 (24.4%) test results were positive for h-FABP. Absolute agreement

between investigators was 93.0%, with a κ score of 0.81 (95% CI 0.6 to 1.0) associated with a p value <0.05 indicating near perfect agreement between investigators. In total, there were three (7.0%) disagreements in test result interpretation. Each of these disagreements appears to have arisen because one observer noted that a result was 'weakly positive' while the other rated the result as 'negative'. In one patient, the discrepancy was noted in the admission blood sample although both observers recorded the result as 'positive' when the 3-hour sample was tested. The other two patients had concordant results for the admission blood samples (both positive in one patient; both negative in the other) but discrepancies were noted at 3 h. As the tests were run on whole blood, we could not directly evaluate the impact of haemolysis in this study. No tests in this study were reported as uninterpretable.

Table 3-2. Baseline characteristics of patients recruited to the interobserver reliability study

Study participants (n= 31)	
Age, mean (standard deviation)	60 \pm 12
Male sex, n (%)	15 (48.4)
Female sex, n (%)	16 (51.6)
Hypertension, n (%)	14 (45.2)
Hyperlipidaemia, n (%)	6 (19.4)
Diabetes mellitus, n (%)	1 (3.2)
Sweating , n (%)	16 (51.6)
Current smoker, n (%)	5 (16.1)
Previous ischaemic heart disease, n	(%) 3 (9.7)
Renal impairment, n (%)	1 (3.2)
Clinicians interpreting test results	
Background of clinician interpreting test result, n (%)	Emergency physician 47 (54.7)
	Nurse (clinical research nurse) 18 (20.9)
	Medical technologist 21 (24.4)

3.4. Discussion

In this study, we have demonstrated that the results of a novel lateral flow immunoassay for h-FABP can be interpreted in clinical settings by staff with various backgrounds with a high degree of interobserver reliability. A (κ) score of 0.81 demonstrates near perfect agreement and the lower boundary of the 95% confidence interval is >0.6 indicating at least substantial agreement. These findings support the use of this test in clinical practice. In this current study we noted that three were discrepancies from our 43 observations, indicating discrepant results in 7% of cases. If results were to be wrongly interpreted as 'negative', this could lead to a missed diagnosis of ACS. However, the test has been calibrated to provide positive results at 4 ng/ml, which is lower than the reported 99th percentile (5.6 ng/ml) of quantitative measurements (Glatz and Mohren, 2013). This has the effect of introducing a 'safety margin' to avoid genuine false negative results. Nowadays, POC tests have the potential to improve health care services and patient outcomes in an emergency setting, especially in triage process. The impact, however, should be robustly evaluated in diagnostic studies prior to implementation. Although h-FABP is not currently widely used, it may help to facilitate early 'rule in' and 'rule out' of acute coronary syndromes in the ED, which would help to reduce unnecessary hospital admissions and identify the highest risk patients at the earliest possible opportunity. H-FABP is incorporated in the MACS decision rule, which has been shown to identify over one quarter of patients as eligible for immediate discharge from the ED with no missed AMIs and a low incidence of major adverse cardiac events within 30 days (Body *et al.*, 2014). Although the MACS rule has been validated using an automated immunoassay for h-FABP, which can be run using commercial laboratory analysers, the requirement to run an assay for an additional biomarker may present a barrier to clinical implementation. A POCT may facilitate clinical implementation. A number of prior studies have evaluated the diagnostic accuracy of h-FABP in chest pain patients suspected of ACS in an emergency department setting (Willemssen *et al.*, 2015b; Slot *et al.*, 2013; Agnello *et al.*, 2017). The lateral flow immunoassay described in this study is a simple and inexpensive technology. It requires only a drop of blood and produces results with a

turnaround time of just 5 min. This novel assay may therefore enable the MACS rule to be used in other settings such as the pre-hospital environment or in the community.

3.5. Limitations of the study

This study does have some limitations. While our approach was pragmatic, the observers in this study had different levels of clinical experience. Each investigator received a standard 2 h training session prior to the study starting but was previously unexperienced to the use of this assay. It is possible that interobserver reliability will improve over time as staff members become more experienced in its use. This study cannot evaluate this approach. This study did not aim to evaluate diagnostic accuracy, which must also be established before the assay can be used in clinical settings. Prior to investing in such work, it was important to evaluate interobserver reliability. And as this study identified suboptimal reliability, the next chapter will evaluate the diagnostic accuracy of this assay in a clinical setting.

**Chapter 4 : Early diagnostic performance of Heart-Fatty Acid Binding
Protein in suspected Acute Myocardial Infraction**

4.1 Introduction

Human Heart Fatty Acid Binding Protein (h-FABP) is a low molecular weight protein (15 kDa) present in cardiac myocytes. It is a transport protein which takes a role in the intracellular uptake and transport of long-chain fatty acids. It is composed of 132 amino acids, and it is one of the most abundant proteins in the myocardial tissue that forms 5-15% of the cytoplasmic proteins in the human heart. Because of the small size and the cytoplasmic (Willemssen *et al.*, 2015a). h-FABP concentrations reach the peak at approximately six to eight hours after symptom onset and back to normal level within twenty-four to thirty hours making it more efficient biomarker for myocardial injury comparing to other cardiac marker (Al-Hadi and Fox, 2009). See Figure 4.1 h-FABP protein can be identified in the blood as early as one to three hours after onset of chest pain, the peak values reached at six to eight hours and plasma levels back to normal within twenty four to thirty hours (Gururajan *et al.*, 2010). Thus, h-FABP appears to be a very stable protein for early detection of myocardial necrosis as studies have shown that serum and plasma samples can be subjected to up to 8 freeze/thaw cycles without the loss of immunoreactivity make it useful for in vitro clinical diagnostic purposes (Wodzig *et al.*, 1997). Prof Glatz (Maastricht University, The Netherlands) was the first to report the diagnostic potential of h-FABP in 1988 (Glatz *et al.*, 1988). Numerous studies have evaluated the added value of h-FABP along with troponin in the ED setting for the early diagnosis of AMI (McCann *et al.*, 2008; Banu *et al.*, 2014; McMahon *et al.*, 2012b). These studies showed higher diagnostic performance of heart fatty acid binding protein when combining with initial cardiac troponin for acute myocardial infarction. In McCann study the sensitivity of h-FABP at initial time was higher than Troponin 76% (69–82), 75% (69–81) and specificity was 61% (55–68) 94% (90–96) respectively. h-FABP test were measured using ELISA test kit in this study. Bannu *et al* and his group were measured h-FABP in POC qualitative device, the researchers found that when combining h-FABP and troponin at initial time the diagnostic sensitivity for AMI was higher than the sensitivity of troponin by its own for the diagnosis of AMI. The sensitivity was 52.78 (35.49-69.59) for the troponin and h-FABP and 50 (32.9-67.1) for troponin only. McMahon group were measured

h-FABP and cTnI, creatine kinase-MB, and myoglobin for the early detection of AMI among patients who present to the ED. The researchers found that when combining cTnI measurement with h-FABP test the sensitivity for AMI diagnosis was increased (71.4% at 3 to 6 hours and 88.2% at 3 to 6 hours) comparing to h-FABP test only where the sensitivity at 0 to 3 hours was (64.3%) and 3 to 6 hours was (85.3%) after chest pain onset. A number of studies have investigated the utility of h-FABP for assisting in the diagnosis of AMI and for prognostic stratification (Jones *et al.*, 2017). A meta-analysis of twenty-two studies including 6602 patients with suspected AMI demonstrate that pooled sensitivity, specificity and AUC of h-FABP were 0.75 (0.68–0.81), 0.81 (0.75–0.86) and 0.85 (0.82–0.88) respectively within six hours. On the other hand, a similar sensitivity (0.76, 0.69–0.82), specificity (0.80, 0.71–0.87) and AUC (0.85, 0.82–0.88) of h-FABP were observed in 4185 (63%) patients at patient presentation and three hours past presentation (Xu *et al.*, 2017). Improving diagnostic protocol to ‘rule out’ ACSs and triage decisions at the time of patient presentation to the emergency department (ED) is essential as the impact of implementing an accurate diagnostic protocol could reduce unnecessary hospital admissions and improve patient outcome. While the imperfect sensitivity and (NPV) of existing diagnostic protocol at the time of patient arrival in ED leads to unnecessary hospital admissions, suboptimal specificity and (PPV) also present challenges. Among patients presenting to the (ED) with suspected ACS, h-FABP has been shown to be an effective independent predictor of major adverse cardiac events (MACE) within 30 days even after accounting for high sensitivity cardiac troponin, electrocardiographic (ECG) changes and clinical information. H-FABP was incorporated in the Manchester Acute Coronary Syndromes (MACS) decision rule (Body *et al.*, 2014). This rule effectively risk stratifies patients using a single blood test, taken at the time of arrival in the ED, to identify subjects who could have ACS immediately ‘ruled out’ (thus avoiding unnecessary hospital admission) and others in whom the diagnosis could be immediately ‘ruled in’. The MACS rule has now been recently validated with an automated laboratory-based h-FABP assay (Body *et al.*, 2014c). However, the requirement to run the h-FABP assay may present a barrier to clinical implementation, as this assay is not routinely used for other indications. As an alternative, h-FABP measured by POC may

possibly be a helpful diagnostic tool. Concerning AMI diagnosis, time is crucial, rapid triaging of chest pain patients presenting to ED is needed to identify positive cases for immediate application of appropriate treatment. The availability of an appropriate test that allows the rapid assessment of H-FABP in ED is beneficial to aid in the diagnosis of AMI. Several point of care testing methods both rapid qualitative or quantitative to determine h-FABP are available in the market (Rhee *et al.*, 2008; Mad *et al.*, 2007a).

Up-To-Date, the performance of h-FABP for acute MI diagnosis has been focused on its comparison with troponin or ECG ischemia see Table 3B.2 for the recent clinical studies that have directly compared h-FABP and troponin applying quantitative or qualitative test in emergency setting. Finally, this chapter is to evaluate the diagnostic accuracy of qualitative (True Rapid, FABPulous BV) POC test in the diagnosis of AMI by its own and in combining with troponin or ECG ischemia. The potential of such a quick device was valuable in several ways, it is a portable qualitative test this was allowing for testing h-FABP at the emergency department next to the patient, and this leading to decreasing the time of patient waiting in ED as this device measuring H-FAPB in 5 minutes. However, this avoids emergency to be overcrowded and avoided unnecessary referrals minimised patients anxiety in addition to extra costs (Yarmohammadian *et al.*, 2017).

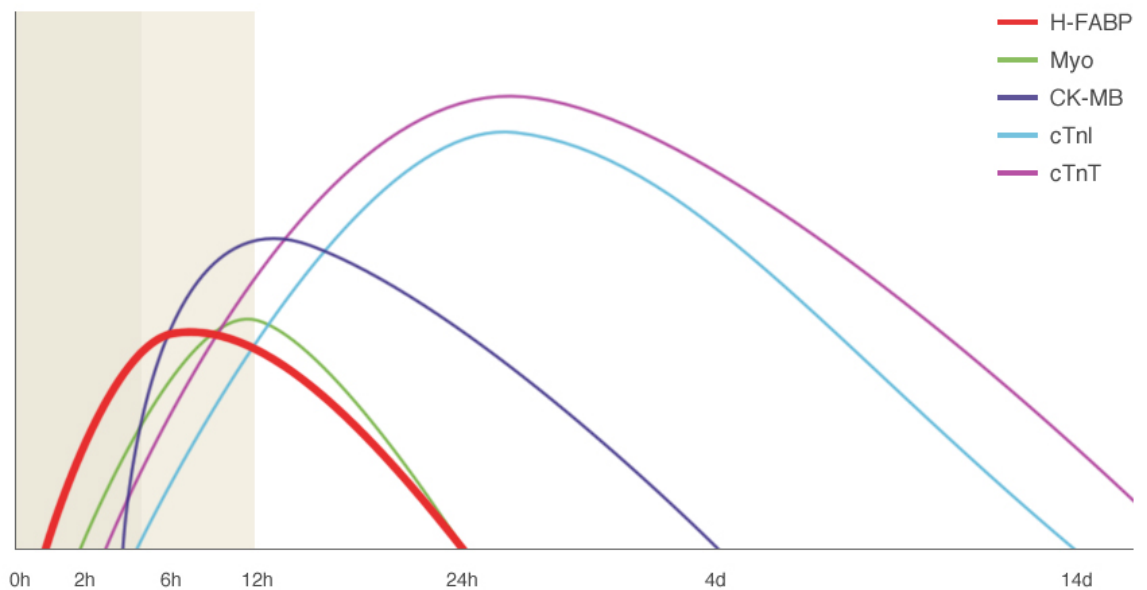


Figure 4-1. h-FABP: Release Kinetic.

h-FABP concentrations reach the peak at approximately six to eight hours after symptom onset and back to normal level within twenty-four to thirty hours. h-FABP has similar release kinetics to Myoglobin but 15 to 20 times more cardiac specific, making it more efficient biomarker for myocardial injury. Moreover, the normal serum or plasma value of h-FABP is much lower than with Myoglobin, which reduces the possibility of false-positive results. Picture adapted from (RANDOX, 2015).

4.2. Aim and Objective

The aim of the current study is to evaluate the diagnostic accuracy of point of care h-FABP lateral flow immunoassay (True Rapid, FABPulous BV) device for diagnosing or excluding AMI using a single test at the time of patient presentation to the ED and three hours later.

4.3. Methodology

4.3.1. Data collection and processing

This chapter reports the preliminary analysis of prospectively observational diagnostic cohort adults who were presented to ED at 14 different hospitals. For this analysis recruitment commenced in February 2015, patients recruited into the study if they met the inclusion criteria of : adults patients (>18 years) who present to the ED with pain, discomfort or pressure in the chest, epigastrium, neck, jaw, or upper limb. Also, patients without an apparent non-cardiac source were this is compatible with the American Heart Association case definitions (Anderson *et al.*, 2007)). Moreover, a patients with peak symptoms occurred <12 hours prior to presentation at the ED. Patients were excluded if they had unequivocal evidence of ST elevation myocardial infarction who are being immediately transferred for primary percutaneous coronary intervention; Patients with another medical condition that would necessitate hospital admission; Patients who lack the capacity to provide written informed consent. Ethical approval and written informed consent were obtained from all subjects.

4.3.2. Measurements

Comprehensive clinical data including were recorded in the case report form at the time of inclusion by the treating physician and study nurse in accordance with contemporary international standards. However, these data including details of: the presenting complaint, previous medical history, medication history and social history (including alcohol intake and tobacco use). In addition to the family history of ischaemic heart disease, findings on physical examination, clinician's impression this was including the clinician's judgement about whether or not AMI is present and also 12-lead ECG findings (including the presence or absence of dynamic ECG changes such as T wave inversion or ST segment depression), medications received were recorded either was it pre-hospital or during the active study phase, disposition; findings of relevant laboratory tests and medical imaging were also recorded. The initial venous blood samples to measure h-FABP were drawn on arrival of patients and three hours later. Fresh whole blood samples were then immediately tested for h-FABP using the FABPulous h-FABP True Rapid Test (FABPulousBV, Maastricht, Netherlands; calibrated to provide a positive result at 4 ng/ml) according to manufacturer's instructions. The h-FABP was evaluated by the researcher, and time of drawing the initial blood sample and all the laboratory measurements were documented. See Figure 4.2. For the appearance look of the positive result for the qualitative FABPulous h-FABP sample.



Figure 4-2. The qualitative FABPulous h-FABP

The top figure is illustrate the positive test in the POC device, both lines (Control and sample) are performs in red colour results was available within 5 minutes.

4.4. Follow up

Patients were followed up throughout their inpatient course and thereafter until 30 days. Data regarding length of stay; cardiac investigations and procedures; details of any haemorrhagic complications.

4.5. Outcome

In this study, two independent investigators (emergency physician and consultant in emergency medicine) defined a composite of acute myocardial infarction (AMI). Both were blinded for the outcome according to the Third Universal Definition of AM (Thygesen *et al.*, 2012b). The reference standard for AMI was based on clinical information and the requirement of patients having a rise and/or fall of serial central laboratory cTn testing over at least 3 hours (for high sensitivity assays) or at least 6 hours (contemporary assays) with at least one troponin level above the 99th percentile within 30 days.

4.6. Statistics

The primary analysis included all participants with complete blood drawn on arrival and 3 hours later. The goal of the primary analysis is to determine the performance characteristics of the FABPulous h-FABP True Raid Test. The

aim of a diagnostic test is to validate the presence or absence of a disease. In this study, The clinical performance of h-FABP as a diagnostic test is completely based on its ability to accurately classify chest pain patients to subgroups (patients with AMI and patients without AMI). In this study the area under the receiver operating characteristic curve (AUC), sensitivity, specificity, (PPV) and (NPV) together with 95% confidence intervals were calculated (using SPSS V.20.0, SPSS, Chicago, Illinois; and/ or MedCalc V.12.4.0.0, Mariakerke, Belgium). However, the basic approach in the calculation of above measures is to presented the result in a 2x2 table with groups of subjects divided according to a gold standard (Sauerbrei and Blettner, 2009) as described earlier in chapter two.

4.7. Result

4.7.1. Diagnostic performance of (FABPulous-FABP) as the only biomarker to rule in and rule out AMI on patient arrival and three hours later

A total of 691 patients were included in this “rule in” and “rule out” study. The h-FABP test results at arrival time to ED were found to be positive for 106 patients (15%) and negative for 585 patients (85%). 44 patients (6%) were incorrectly classified as not having AMIs (false negative result). those are the patients who has negative results and having AMI (misclassified patients as false negative). Results are summarised in Table 4.1. The sensitivity and NPV to rule out AMI were 48.24% (95%CI: 37.26% to 59.34%) and 92.48 % (95%CI: 90.91% to 93.80%) respectively. While Specificity and PPV were 89.27 % (95%CI: 86.53%to 91.62%) and 38.68% (95%CI: 31.45% to 46.44%) respectively Table 4.2. This strategy would allow 85 % of patients to be discharged (rule out percentage). In contrast at three hours post arrival, 663 patients were included in the study for h-FABP analysis. The results of h-FABP test revealed positive for 89 patients (13%) and negative for 574 patients (86%). 32 patients (4.8%) were incorreced classification with missing AMIs (false negative result). see 4.3 and 4.4. Tables

Table 4-1. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the FABPulous h-FABP True Raid Test only decision aid at patient presentation.(n= 691)

Performance of h-FABP		AMI	No AMI	Total
h-FABP at initial Time (Time 0 on patient presentation to ED)	Test Positive	TP 41	FP 65	(TP + FP) 106
	Test Negative	FN 44	TN 541	(FN + TN) 585
Total		(TP+FN) 85	(FP + TN) 606	(TP+TN+FP+FN) 691

TP: True positive, FP: False positive, FN: False negative, TN: True negative.

Table 4-2. Significance of diagnostic Rule-out strategy of FABPulous h-FABP at patient presentation on patient arrival

Diagnostic accuracy of the rule out strategy at patient presentation on patient arrival	Value, %	95% CI	Percentage 'ruled out' AMI
Sensitivity	48.24%	37.26% to 59.34%	585/691=84 %
Specificity	89.27 %	86.53%to 91.62%	
PPV	38.68%	31.45% to 46.44%	
NPV	92.48 %	90.91% to 93.80%	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI : FN+TN/ (TP+TN+FP+FN).

Table 4-3. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the FABPulous h-FABP True Raid Test only decision aid with at three hours post arrival (n= 663)

Performance of h-FABP		AMI	No AMI	Total
h-FABP at 3 hours post arrival	Test Positive	TP 48	FP 41	TP + FP 89
	Test Negative	FN 32	TN 542	(FN+TN) 574
Total		(TP+FN) 80	(FP+TN) 583	(TP+TN+FP+FN) 663

h-FABP at 3 hours post arrival: sample taken 3 hours post arrival, TP: True positive, FP: False positive, FN: False negative, TN: True negative.

Table 4-4. Significance of diagnostic Rule-out strategy of FABPulous h-FABP at Three hours post presentation post arrival

Diagnostic accuracy of the rule out strategy at three hours post arrival	Value%	95% CI	Percentage 'ruled out' AMI
Sensitivity	60.00%	48.44% to 70.80%	574/663=86.5%
Specificity	92.97%	90.58% to 94.91%	
PPV	53.93%	45.33% to 62.31%	
NPV	94.43%	92.83% to 95.68%	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI: FN+TN/ (TP+TN+FP+FN)

4.7.2. Diagnostic performance of FABPulous h-FABP True Raid Test and troponin to rule in and rule out) AMI on patient arrival

A total of 742 patients were included in this “rule in” and “rule out” AMI strategy. H-FABP and i-STAT troponin test cut off at the analytical sensitivity of the cTnI method (>0.02 ng/mL) which is the lowest cTnI level that can be distinguished from zero were found to be positive for 141 patients (19%) and negative for 601 patients (80%). 101 patients (36%) those are the patients who has negative results and having AMI (misclassified patients as false negative). Results are summarised in Table-3a. The sensitivity and NPV to rule out AMI were 39.16% (95%CI: 31.69% - 47.02%) and 83.19 % (95%CI: 81.36%-84.89%) respectively. While Specificity and PPV were 86.81 % (95%CI:

83.77%-89.46%) and 46.10% (95%CI: 39.20%- 53.15%) respectively (Table 4.5 and 4.6. However, this strategy would allow 80 % of patients to be discharged.

Table 4-5. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for rule-out and rule- in myocardial infarction on arrival and three hours later of patient arrival for h-FABP combined with i-STAT >02ng/m

Performance of h-FABP		AMI	No AMI	Total
Maximum h-FABP+i-STAT> 02ng/mL	Test Positive	TP 65	FP 76	TP+FP 141
	Test Negative	FN 101	TN 500	(FN+TN) 601
Total		(TP+FN)166	(FP+TN) 576	(TP+TN+FP+FN) 742

Maximum h-FABP: Maximum h-FABP on arrival and 3 hours later is the highest FABPulous h-FABP recorded on arrival and three hours later, TP: True positive, FP: False positive, FN: False negative, TN: True negative

Table 4-6. Significance of diagnostic Rule-out strategy of FABPulous h-FABP and i-STAT result on arrival and three hours later.

Diagnostic accuracy of the rule out strategy	Value%	95% CI	Percentage 'ruled out'
Sensitivity	39.16%	31.69%-47.02%	601/742= 80%
Specificity	86.81 %	83.77%-89.46%	
PPV	46.10%	39.20%-53.15%	
NPV	83.19 %	81.36%-84.89%	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI: FN+TN/ (TP+TN+FP+FN)

4.7.3. Diagnostic performance of FABPulous h-FABP True Raid Test combine with ECG interpretation and i-STAT troponin result to 'rules in' and 'rules out' AMI strategy

A total of 702 patients were included in this "rule in" and "rule out" AMI strategy. h-FABP, ECG ischemia and i-STAT troponin tests resulted positive in 84 patients (11%) and negative in 292 patients (41%). 2 patients (0.2%) were missing AMIs (false negative result). The results are summarised in Tables 4.7 and 7.8. The sensitivity and specificity to rule out AMI were 97.67% (95%CI: 91.85% -99.72%) and 47.40 % (95%CI: 43.40% -51.43%) respectively. Positive predictive value and negative predictive value were 20.59%

(95%CI:19.28%-21.96%) and 99.32% (95%CI: 97.37% -99.83%) respectively. This strategy would allow 41% of patients to be discharged.

Table 4-7. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infraction on arrival and three hours later of patient presentation

Rule out strategy		AMI	No AMI	Total
Maximum h-FABP on arrival and 3 hours later+ECG interpretation + maximum i-STAT <0.01 µg/L Strategy	Test Positive	TP 84	FP 324	(TP+FP) 408
	Test Negative	FN 2	TN 292	(FN+TN) 294
Total		(TP + FN) 86	(FP+TN) 616	(TP+TN+FP+FN) 702

Maximum h-FABP on arrival and 3 hours later: The highest FABPulous h-FABP recorded on arrival and three hours later, Maximum i-STAT result: The highest i-STAT troponin recorded, ECG interpretation: ECG shows acute ischaemia.

Table 4-8. Significance of diagnostic Rule-out strategy of FABPulous h-FABP and ECG interpatient and i-STAT result on arrival and three hours later

Diagnostic accuracy of the rule out strategy	Value%	95% CI	Percentage 'ruled out'
Sensitivity	97.67%	(91.85%-99.72%)	294/702=41%
Specificity	47.40%	(43.40%-51.43%)	
PPV	20.59%	(19.28%-21.96%)	
NPV	99.32%	(97.37%-99.83%)	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI: FN+TN/ (TP+TN+FP+FN)

4.4. Discussion

h-FABP is a critical diagnostic biomarker in assessing patients for ACS (Mueller, 2014). In the current study, we have investigated whether it is possible to safely rule out AMI using FABPulous h-FABP True Raid Test as the only biomarker strategy measured at the time of patient arrival in ED with possible cardiac chest pain as well as in combination with ECG and i-STAT troponin results. The availability of an appropriate test that allows the rapid assessment of elevated marker concentrations in plasma is essential to support the diagnosis of MI Measurement. The diagnostic sensitivity of h-FABP to rule out AMI by its own was higher three hours post arrival of patients comparing to the initial time sensitivity. However, the obtained results revealed lack of sensitivity of single h-FABPulous h-FABP test for diagnosis of AMI on patient arrival and three hours later with 48.24% and 60% sensitivity). These results are in line with previous finding from a diagnostic study, where the researchers used a) similar POCT at patient arrival and three hours later for chest pain patients attending ED with 3 hours of chest pain onset. The sensitivity of h-FABP test alone at patient arrival and 6 hours after admission was 54%, 71% and 67% respectively (Verbrugge *et al.*, 2015). One of the previous study on use of FABPulous device in diagnosing AMI on ED population reported sensitivity values of 57% at 3 hours, which increased to 75% overall (Buntinx, 2013). h-FABP could be used in combination with or as an alternative of troponin in the current clinical pathway to aid early diagnosis of AMI. Our findings suggest that adding the h-FABP as a part of multi-marker strategy including i-STAT and ECG interpretation can be used for early exclusion of AMI where the sensitivity of this strategy was increased to 97.67% with only two patients missing AMIs. This strategy would allow 41% of patients with suspected cardiac chest pain to be immediately rule out and can be discharged from the hospital.

Increasing number of studies have shown that h-FABP is a sensitive marker for the diagnosis of myocardial infarction. Several researchers have evaluated the diagnostic accuracy of h-FABP over myoglobin and troponin and found that at the first hour of myocardial injury, h-FABP has sensitivity equal to that of CK-MB and superior to that of myoglobin. (Gami *et al.*, 2015; Pyati *et al.*,

2016; Body *et al.*, 2011a). It has been reported that h-FABP has superior sensitivity to contemporary cardiac troponin assays when measured at presentation in early presenters (<4h from symptom onset). A study by McMahan *et al.* (2012) evaluate troponin, CK-MB, Myoglobin and h-FABP at different windows time of chest pain onset, and found that the optimum combination for the diagnosis of AMI across the different time points was troponin and h-FABP together. However, the researcher strategy offered a negative predictive value (NPV) of 98% for the exclusion of AMI at just 3-6 hours after chest pain onset. (McCann *et al.*, 2008; McMahon *et al.*, 2012a). Access to immediate cardiac biomarkers could facilitate early diagnosis of ACS. However, When it comes to AMI diagnosis, time is crucial, hence with rapid reduction in processing time of patient triage and shorter time to treatment are strongly linked to better survival (Hand, 1994). Much of the available literature and research evaluate the diagnostic accuracy of h-FABP on laboratory test. Point-of-care blood tests reduce the time taken for the result to be available for emergency physicians compared to laboratory blood tests, allowing definitive treatment to be started faster (Amundson and Apple, 2015). When measuring h-FABP obtaining a false negative result could cause a treating physician to miss the diagnosis of ACS, leading to a potentially dangerous outcome. False positive results, on the other hand, could lead to unnecessary patient distress and excessive interventions with their associated risks and costs (Lum *et al.*, 2006). The sensitivity of another POC device for h-FABP test was reported in several studies. However, the sensitivity ranged widely from 8.7% to 98%, with a median value of 71% (Figiel *et al.*, 2008; Cavus *et al.*, 2006; Valle *et al.*, 2008). New systematic reviews and meta-analyses studies on the combination of h-FABP and high sensitivity troponin showed sensitivity, specificity and AUC of h-FABP to be 0.75 (0.68-0.81), 0.81 (0.75-0.86) and 0.85 (0.82-0.88) within six hours. Conversely, similar sensitivity (0.76, 0.69-0.82), specificity (0.80, 0.71-0.87) and AUC (0.85, 0.82-0.88) of h-FABP were observed in 4185 (63%) patients in 0h/3h algorithm. The additional use of h-FABP improved the sensitivity of hs-Tn alone but no increase in AUC with declined specificity (all $p < 0.001$) (Xu *et al.*, 2017). Some of the studies have reported improved detection and exclusion of AMI with early h-FABP test when compared with testing of cardiac troponin alone

(Ecollan *et al.*, 2007; Body *et al.*, 2011a; Keller *et al.*, 2011b). Recently, a systematic review on four clinical studies (Mion *et al.*, 2007; McCann *et al.*, 2008; Haltern *et al.*, 2010; Body *et al.*, 2011a) has shown that the addition of h-FABP test to troponin increased sensitivity from (42–75 %) to (76–97 %) but decreased specificity from (95–100 % to 65–93 %). One of the previous work revealed that POC offers sensitivity of 92% after 3 hours of symptom onset and NPV of 99% when used in conjunction with ECG. The sensitivity was higher for AMI diagnosis when results were combined with ECG) (Glatz and Renneberg, 2014).

Diagnostic strategies is needed to overcome the current sensitivity deficit. In general, FABPulous h-FABP True Raid Test did not provide high sensitivity when it is used by its own. Previously, a computer model the Manchester Acute Coronary Syndrome (MACS) algorithm used a laboratory-based h-FABP assay test, which can be run with commercially available analysers in hospital laboratories (Body *et al.*, 2014b). This strategy required a new assay to be set up and operated by the laboratory staff, which is a barrier to clinical implementation. However, the present study offers further insight into potential use of FABPulous h-FABP True Raid Test (FABP-ulous, Netherlands), which gives the necessary clinical evidence to support the adoption as a way for emergency medical workers to rule out heart attacks before they are required to admit patients to hospital. The test provides dichotomous (positive/negative) results within 5 minutes and can be used at the patient site in ED.

Strengths and limitation

The current study applied the FABP True Rapid Test which is a whole blood medical device that is designed to provide a reliable measurement of the Heart-type Fatty Acid Binding Protein (h-FABP). To our knowledge this is the first study that evaluated this device in multicentre study with inclusion of a wider range of population group. In addition, the device used in the current study was meeting the demand of a lower cut-off value; the cut off value of this device was 4 ng/ml which was found to be the lower in the market to date.

**Chapter 5 : Improved sensitivity of point- of- care troponin I values
using reporting the limit of detection and functional sensitivity to
rapidly rule out myocardial infarction early results of the Bedside
Evaluation of Sensitive Troponin (BEST)**

5.1. Background

Achieving a rapid and accurate diagnosis of acute myocardial infarction (AMI) is essential, since early intervention save lives. The diagnosis of AMI is based on several criteria, including observation of circulating cardiac troponin I or T (cTnI or cTnT) above the upper reference limit (URL) (Jaffe and Apple, 2012a). Immediate rule-out of AMI in patients presenting with symptoms of cardiac ischaemia facilitates early discharge of this group of patients helps to reduce overcrowding of emergency departments (ED) (Taylor *et al.*, 2002). Current guidelines support the use of sensitive cTn assays in decision making of AMI diagnosis at admission and three hours post admission of patients (Roffi *et al.*, 2015). In most of the recently published data several rule out protocols were based on the use of high-sensitive (hs) cTnT or cTnI assays (Shah *et al.*, 2015; Bandstein *et al.*, 2014; Tobias Reichlin *et al.*, 2015; Body *et al.*, 2015b). The time needed for results to become available for emergency physicians from the hospital central laboratory usually delays the disposition decision for an additional one or two hours (Bingisser *et al.*, 2012). The routine scenario in clinical practice is such that, when patients arrive at the ED with a complaint of chest pain, they are immediately subjected to a first blood test at time zero. Alternatively, this could be delayed to half an hour and second test could be taken three hours later, so that there is a time of three and half hours after patient arrival. The blood samples are then sent to the central laboratory for tests, which will take another hour or more. Now with a four hours processing target it is impossible to get reports of two tests with a turnaround time of one hour. The only way to get the results of a three hour sample for diagnosing the patient within the four -hour target is to analyse the troponin sample by point of care testing keeping in view very short turnaround time (TAT). A large and growing body of literature has investigated the performance and analytical accuracy of several laboratory troponin assays. However, an assay with high diagnostic sensitivity is inevitably associated with detecting troponin and the diagnosis of ACS (Bhoi *et al.*, 2014). Point-of-care (POC) assays have the potential to improve the diagnosis of chest pain patients in ED, by delivering quick test results of cTn. The biggest advantage of point of care tests when compared with central laboratory is shorter turnaround time that saves time in

the range of 47 to 59 minutes (Venge *et al.*, 2010; McCord *et al.*, 2001). However, this advantage is at the cost of lower diagnostic accuracy and lower negative predictive value. At this time, the majority of the available (POC) assays cannot be considered sensitive or high sensitivity assays (Apple, 2009). Currently, cTnI tests by POC display a lower diagnostic accuracy than central laboratory cTnI/T assays (Apple *et al.*, 2012b). The consensus statement on the universal definition of myocardial infarction recommends that an increase in plasma troponin concentration above the 99th percentile of a normal reference population is used to confirm the diagnosis (Jaffe and Apple, 2012b). Despite this recommendation, lowering the diagnostic threshold for troponin remains a challenge whether the benefits of improved sensitivity will overcome the problems that might arise from reduced specificity (Kavsak and Jaffe, 2009). Accelerated diagnostic protocols that apply a high-sensitivity troponin level below the level of detection (LoD) have been explored and give a promising tool for discharge at the time of patient arrival to the ED. Numerous observational studies have evaluated the (LoD) (lowest analyte concentration at which detection is feasible) of hs-cTn assays alone at presentation to ED (Body *et al.*, 2015c; Carlton *et al.*, 2016). In the literature, both the limit of blank (LoB) and limit of detection (LoD) have been used as thresholds to define detectability. In a recent meta-analysis of more than 9000 chest pain patients in ED from 11 comprehensive studies, the researchers have estimated the diagnostic accuracy of a single hs-cTnT concentration below the (LoD) in addition to a non-ischemic electrocardiogram (ECG). The researchers found out that 30.6% were classified as low risk and pooled sensitivity for 30-days major adverse cardiac events (MACEs) was 98.0% (95% CI 94.7% to 99.3%). They concluded that the LoD cut-off concentration of the hs-cTnT assay, when combined with a non-ischaemic ECG, is actually safe for rule out AMI in patients presenting to EDs with possible emergency acute coronary syndrome (Pickering *et al.*, 2017).

(POC) (cTn) assays are not 'high sensitivity' but some can detect cTn concentrations below the conventional 99th percentile cut-off. There is limited evidence for the diagnostic accuracy and clinical sensitivity of detecting AMI at patient's presentation to ED. The Abbott i-Stat handheld POC assay

provides a cTnI result within 10 min. However, there is some evidence for the i-STAT at 3 hours but it is not good enough to rule out the patients based on that strategy. The existing evidence for the diagnostic accuracy of POC cTn testing is well known to be limited. There is currently no evidence that explores the cut-off set at the lower limit of detection of POC testing in ruling out chest pain patients in EDs.

5.2. Aim and objectives

The primary aim of this present study was to determine the diagnostic accuracy of the Abbot *i-Stat* handheld POC assay. The study was also aimed to determine whether serial troponin testing using the Abbott *i-Stat* POC troponin device can accurately 'rule in' and/or 'rule out' the diagnosis of (AMI) within 3 hours of arrival in the ED, using either the conventional 99th percentile cut-off or a novel 'rule out' cut-off set at either (a) the limit of detection of the assay, or (b) the lower limit of the reportable range of the assay.

The objectives of this study were to address the following research questions: In patients with suspected acute coronary syndromes can serial troponin testing within 3 hours of arrival in the ED using the Abbott *i-Stat* point of care device enable accurate identification of:

- a. Patients at sufficiently low risk of prevalent AMI, to enable safe avoidance of hospital admission.
- b. Patients at sufficiently high risk of prevalent AMI so that that the diagnosis of an ACS should be considered 'ruled in'.

5.3. Materials and methods

5.3.1. Data collection and processing

In this study, we prospectively enrolled an adult population presenting to the ED at eighteen hospitals with suspected ACS. All patients who were included in the study were entered into this diagnostic analysis. Patients with unequivocal evidence of ST elevation myocardial infarction requiring immediate primary percutaneous coronary intervention (PCI) were excluded from the study. The full inclusion and exclusion criteria have been described in detail in section 2.1.3. Comprehensive clinical, electrocardiographic and biochemical data were collected at the time of presentation using a CRF form (Appendix 2.3). The initial ECG findings and systolic blood pressure was recorded at the time of arrival in the ED.

5.3.2. Interventions

Subjects who consented and met the inclusion criteria of the study underwent venipuncture within 1 hour of arrival in the ED (or within 45 minutes of the collection of the first blood sample for clinical purposes). Venous whole blood was collected in lithium heparin and EDTA blood tubes at the following intervals: upon enrolment at presentation and three hours later. cTnI concentrations were analysed using the (Abbott Point of Care, New Jersey, 99th percentile 0.08 ng/mL ($\mu\text{g/L}$), LoD 0.02 ng/mL, 0.10 ng/mL, and functional sensitivity at 10% CV).

POC testing was undertaken immediately following venipuncture by a clinical research nurse or the author. Tests were undertaken in accordance with manufacturer's instructions. After the analysis, the time and result were recorded in the participant's case report form. As part of the patient's routine clinical care, all patients were subjected to reference standard laboratory-based troponin testing in accordance with the latest national and international guidance. Additional samples were stored in SST II Advance, lithium heparin and EDTA vials and labelled with a unique study number. After processing any samples that were tested in real time using point of care devices, the additional samples was centrifuged within 30 minutes of venepuncture at 2,500G for 10

minutes. Plasma and serum were separated and aliquots of 500 to 2,000µL and then stored in vials labelled with the patient's unique study number. Sample characteristics (haemolysis, lipaemia, icterus) were also recorded in the case report form. The serum and plasma were then frozen at or -20°C or below within 4 hours of sample collection. The sample collection schedule has been described earlier in (Chapter 2) and the overall schedule for blood sampling during this study is outlined in section 2.1.4.

The reference standard for AMI was serial central laboratory cTn testing over at least 3 hours (for high sensitivity assays) or at least 6 hours contemporary assays), the time window of blood sampling was set according to the local hospital standard procedures of each sites. A blood sample was collected and sent to the laboratory for cTn testing. The diagnostic evaluation and patient management continued as per the standards of the treating hospital system without intervention per study protocol. Cardiac events and any procedures were recorded during the index hospitalization. A 30-day telephonic follow up and by medical record review was carried out. All diagnoses of AMI were performed according to the third universal definition of AMI guidelines (Jaffe, 2013).

5.3.3. Outcome measuring and defining outcomes

The primary outcome of this study was rapid diagnosis AMI. Outcomes were adjudicated by two independent investigators with reference to relevant clinical information but blinded to the results of research investigations. Discrepancies were resolved by consultation with a third independent investigator. AMI was defined according to the Third Universal Definition of AMI (Jaffe, 2013) however, by the inclusion criteria, all patients has symptoms and signs consistent with myocardial ischaemia. Therefore, patients who were deemed to have met this outcome if they developed a rise and/or fall of troponin to above the 99th percentile. At the time of writing this thesis and pending further evidence and international guidance, a significant rise and/or fall of troponin was considered to be >9.2ng/L for high sensitivity troponin T (Mueller *et al.*, 2012) and at least 20% for all other assays.

5.3.4 Statistical methods

In this study we assessed the overall diagnostic accuracy of (i-Stat, Abbott Point of Care) and hs-cTnT by ROC curve analysis with SPSS version 20.0. Diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratios were calculated at the standard (99th percentile cut-offs 80ng/L) and at the LoD of the assay (LoD 0.02 ng/mL) and at the functional sensitivity (10ng/L) of the assay (0.01 ng/mL ($\mu\text{g/L}$)). All statistical analyses were performed using either SPSS version 22.0 (IBM Software, USA) or MedCalc online calculator version Construct 2x2 table at 0.02 $\mu\text{g/mL}$ and 0.08 $\mu\text{g/mL}$ for AMI diagnosis (crosstab by SPSS) (2x2 table).

5.4 Result

5.4.1. Study population

During the study time frame of February 2015 to March 2017 there were 1,613 patients enrolled. Of this cohort, some patients were excluded for missing i-Stat on arrival, and the patients who did not have an ECG recorded were also excluded. This left 733 patients in the final group for analysis consisting of 457 men (62.3%) and 276 women (37.7%). The mean age was 58 years (standard deviation 16). Acute myocardial infarction (AMI) was diagnosed in 90 (12.3%) patients.

5.4.2 Receiver operating characteristic (ROC) curve for the i-STAT troponin assay at presentation, for diagnosis of AMI in the total study population.

As an illustration, the corresponding empirical ROC curve was drawn in Figure (4.1 a,b) by a nonparametric method using SPSS software which shows the ROC curves for diagnosis of AMI with each time point presented in the total study population. At patient presentation, the ROC AUCs was 0.870, and standard error was 0.025 while at three hours later (maximum relative change) of patient presentation the AUCs was 0.910, and standard error was .021. There was significant improvement in the AUCs for the maximum change of troponin. The calculation of sensitivity and specificity of various cut-off values of i-STAT troponin for diagnosis of AMI at the initial time and three hours later of patient presentation are shown in Tables 5.1 and 5.2. Via from the ROC curve, the optimum cut-off value for the diagnosis of AMI (Figures 5.1 and 5.2) appeared to be at 0.005 µg/L, which corresponded to a sensitivity of 87% and a specificity of 69.7%. Conversely, at the LoD 0.02 µg/L the sensitivity was 62.8%, and the specificity was 94% to rule out AMI, which is a higher diagnostic accuracy than the 99th percentile cut off 0.08 µg/L that corresponded to a sensitivity of 45% and specificity of 98%. Although the i-Stat has higher specificity, it would not be the better choice of the two cut-offs for ruling in.

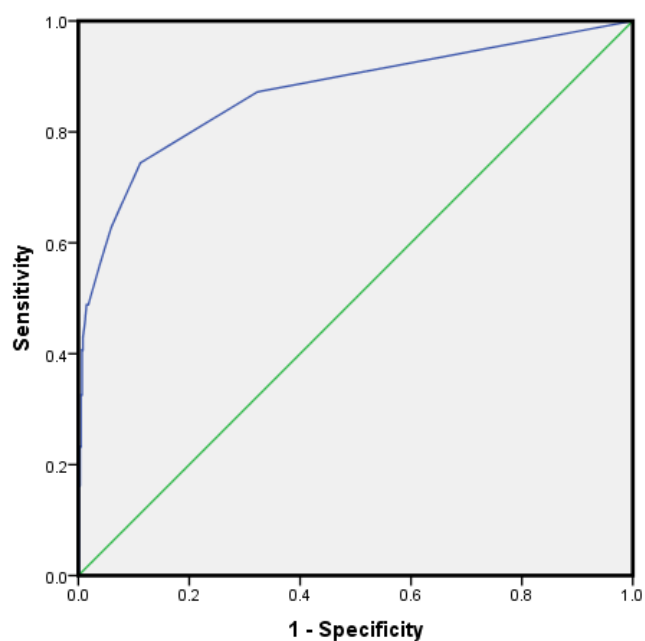


Figure 5-1. Receiver operating characteristic (ROC) curve for the i-STAT troponin assay at presentation, for diagnosis of AMI in the total study population

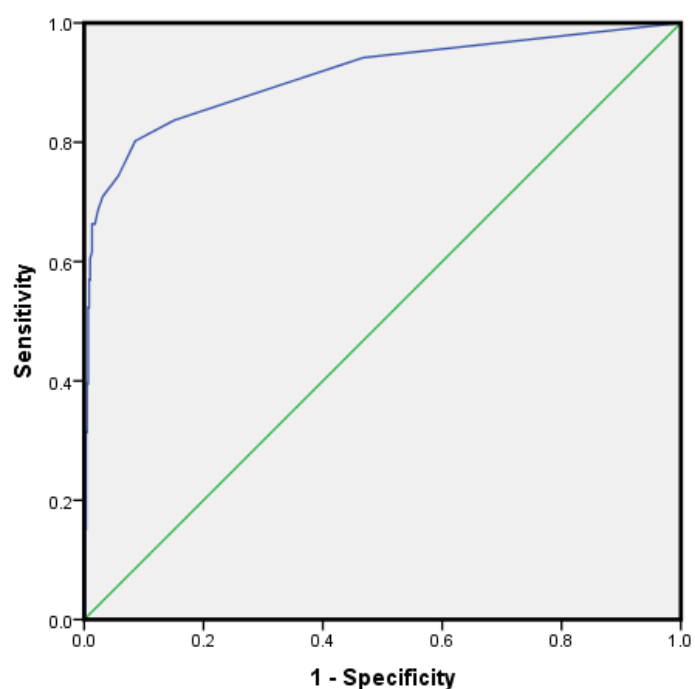


Figure 5-2. Receiver operating characteristic (ROC) curve analysis for the i-STAT troponin assay measured at three hours after presentation in the total study population, for a diagnosis of AMI

Table 5-1. Optimal cut off-values of i-STAT at each cut-off value at initial time for AMI with 95% CI

Optimal cut-off-value (ng/mL)	Sensitivity%	Specificity%	95% AUC	
			Lower Limit	Limit-Upper
0.005 µg/L	87%	69.7%	0.870	0.821-0.919
0.02 µg/L	62.8%	94%		
0.08 µg/L	45%	98%		

Area under the Curve (AUC): The c-statistic or AUC, is derived from receiver-operating characteristic curves (ROC) and is used to determine the discriminatory ability of a diagnostic test. The AUC equals 0.5 when the diagnostic test corresponds to random chance (null hypothesis), and 1.0 shows perfect diagnostic accuracy. Note: definition reproduced from Hajian Tilaki with permission.

Table 5-2. Optimal cut off-values of i-STAT at each cut-off value at three hours later

Optimal cut-off-value (µg/L)	Sensitivity%	Specificity%	AUC (95% CI) (Lower Bound-Upper Bound)
0.005 µg/L	94%	54%	0.910 (0.869-0.952)
0.02 µg/L	80%	84%	
0.08 µg/L	65%	99%	

Abbreviations: AUC, area under the curve.

5.4.3 Performance of the i-STAT POC assay at the limit of detection .02 ng/mL (µg/L) (LoD, 20ng/L)

A total of 693 patients were included in this analysis, of which 86 (12.4%) patients had AMI. The proportion of patients had AMI are summarised in Table 5.3. (2x2 Table) However, with this rule out strategy at LoD (0.02 µg/L) the diagnostic sensitivity to rule out AMI was 62.79% (95% CI 51.70% - 72.98%) and a negative predictive value of 94.69 % (95% CI 93.13% -95.92%). This cut-off would miss the diagnosis of 32 (4.6%) patients. On the other hand, the diagnostic specificity was 94.07 % (95% CI 91.88% -95.81%) and positive

predictive value of was 60.00% (95% CI 51.23%-68.17%). The diagnostic sensitivity at lower limit of detection cut-off value (0.02 ng/ml) to rule out AMI three hours after patient arrival to ED was 83.72% (95% CI 74.20%-90.80%) and a negative predictive value of 97.37 % (95% CI 95.81% to 98.36%). On the other hand, the diagnostic specificity was 84.92 % (95% CI 81.83% to 87.67%) and the positive predictive value was 43.90% (38.81%-49.12%). The results are summarised in Table 5.4, 5.5 and 5.6.

Table 5-3. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival using the 0.02 ng/ml cut-off

		AMI	No AMI	Total
Performance of the i-STAT POC assay at the lower limit of detection 0.02 ng/ml	>0.02 ng/ml	TP 54	FP 36	90
		FN 32	TN 571	603
Total		86	607	693

i-STAT T0 at 0.02 ng/mL : i-STAT troponin result at initial time , TP: True positive, FP: False positive, FN: False negative, TN: True negative.

Table 5-4. Rule-out strategy, POC cTn<0.02 ng/ml on arrival

Diagnostic performance measure	test	Value,%	95% Confidence Interval Lower Limit-Upper Limit	Percentage 'ruled out' (Total FN+TN)/100
Sensitivity		62.79%	51.70% - 72.98	603/693 x100 87%
Specificity		94.07 %	88% -95.81	
PPV		60.00%	51.23-68.17	
NPV		94.69 %	93.13 - 95.92	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI : FN+TN/ (TP+TN+FP+FN) X100

Table 5-5. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction at three hours later of patient presentation using the 0.02 ng/mL cut-off

		AMI	No AMI	Total
Max i-STAT 0.02 ng/mL	>.02 ng/mL	TP 72	FP 92	164
		FN 14	TN 518	532
Total		86	610	696

Max i-STAT 0.02 ng/mL (µg/L): The highest i-STAT troponin recorded on arrival & 3 hours TP: True positive, FP: False positive, FN: False negative, TN: True negative

Table 5-6. Rule-out strategy, POC cTn<0.02 ng/mL on arrival & 3 hours post arrival

Diagnostic test performance measure	Value,%	95% Confidence Interval, Lower Limit-Upper Limit	Percentage 'ruled out'
Sensitivity	83.72%	74.2-90.80	532/696×100 76.4%
Specificity	84.92 %	81.83 87.6	
PPV	43.90%	38.8 -49.12	
NPV	97.37%	95.8 -98.36	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI : FN+TN/ (TP+TN+FP+FN) X100

5.4.4 Performance of the i-STAT POC assay at the conventional 99th percentile cut-off 0.08 ng/mL

With this rule out strategy at the 99th percentile cut-off value (0.08 ng/ml) at initial i-STAT on arrival, 42 patients had AMI (5 patients had false positive result) see Table 5.7. The diagnostic sensitivity to rule out AMI was 43.02% (95% CI 32.39% 54.15%) and a negative predictive value of 92.47% (95% CI 91.09% to 93.66%). Using this diagnostic cut-off would miss 49 (7.6%) patients. Conversely, the diagnostic specificity was 99.18 % (95% CI 98.09%-99.73%) and positive predictive value of 88.10% (95% CI 74.94%-94.82%). The results are summarised in Tables 5.8, 5.9 and 5.10

Table 5-7. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction at arrival of patient presentation using the 0.08 ng/ml the conventional 99th percentile cut off

		AMI	No AMI	Total
Troponin i-STAT at<0.08 ng/ml on arrival	<0.08 ng/ml	TP 37	FP 5	42
		FN 49	TN 602	651
Total		86	607	693

TP: True positive, FP: False positive, FN: False negative, TN: True negative, <0.08 ng/ml:the conventional 99th percentile cut-off.

Table 5-8. Rule-out strategy, POC cTn 0.08, ng/ml the conventional 99th percentile cut-off on arrival

Diagnostic test performance measure	Value, %	95% Confidence Interval Lower Limit-Upper Limit	Percentage 'ruled out'
Sensitivity	43.02%	32.39%- 54.15%	651/693×100=94%
Specificity	99.18 %	98.09% to 99.73%	
PPV	88.10%	74.94% to 94.82%	
NPV	92.47 %	91.09% to 93.66%	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI: $FN+TN / (TP+TN+FP+FN) \times 100$

Table 5-9. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival & 3 hours later of patient presentation using the 0.08µg/L cut-off

	AMI	No AMI	Total
Troponin i-STAT <0.08 ng/ml on arrival & 3 hours	TP 56	FP 8	64
	FN 30	TN 602	632
Total	86	610	696

TP: True positive, FP: False positive, FN: False negative, TN: True negative, 08 ng/ml the conventional 99th percentile cut-off

Table 5-10. Rule-out strategy, POC cTn 08 ng/ml on arrival & 3 hours post arrival

Diagnostic test performance measure	Value, %	95% Confidence Interval Lower Limit-Upper Limit	Percentage 'ruled out' (Total FN+TN)/100
Sensitivity	65.12%	54.08% to 75.08%	632/696× 100=90%
Specificity	98.69 %	97.43% to 99.43%	
PPV	87.50%	77.56% to 93.41%	
NPV	95.25 %	93.76% to 96.40%	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI: $FN+TN / (TP+TN+FP+FN) \times 100$

5.4.5 Performance of i-STAT POC assay at the functional sensitivity (10ng/L) of the assay (0.01 ng/mL

With this rule out strategy at the functional sensitivity of the assay at patient presentation the diagnostic sensitivity to rule out AMI using this cut-off was 94.19% (95% CI 86.95% - 98.09%) and a negative predictive value of 98.48 % (95% CI 96.51%- 99.35%). However, using this diagnostic cut-off would miss diagnosis of five (.8%) patients. Conversely, the diagnostic specificity was 53.28 % (95% CI 49.23% to 57.30%) and positive predictive value of 98.48 % (95% CI 96.51%-99.35%). The results are summarised in Tables 5.11 and 5.12. Summary of the diagnostic accuracy specification for i-STAT cardiac troponin assay for ruling out AMI at different cut offs are illustrated in Table 5.13

Table 5-11. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival and three hours later of patient presentation using the functional sensitivity

		AMI	No AMI	Total
i-STAT<0.01 ng/mL (µg/L).on arrival & 3 hours	<0.01 ng/ml	TP 81	FP 285	366
		FN 5	TN 325	330
Total		86	610	696

TP: True positive, FP: False positive, FN: False negative, TN: True negative, <0.01 ng/ml: the functional sensitivities for the cTnl method

Table 5-12. Rule-out strategy, POC cTnat the functional sensitivity of the assay <0.01 ng/ml on arrival & 3 hours post arrival

Diagnostic test performance measure	Value,%	95% Confidence Interval Lower Limit-Upper Limit	Percentage 'ruled out'
Sensitivity	94.19%	86.95% to 98.09%	330/369 x100 89.5%
Specificity	53.28 %	49.23% to 57.30%	
PPV	22.13%	20.46% to 23.90%	
NPV	98.48 %	96.51% to 99.35%	

PPV: Positive predictive value, NPV: Negative predictive value, Percentage 'ruled out' AMI: FN+TN/ (TP+TN+FP+FN) X100

Table 5-13. Summary of the diagnostic accuracy specification for i-STAT cardiac troponin assay for ruling out AMI at different cut offs

Rule-out strategy, POC cTn	Percentage 'ruled out'	Sensitivity%	Specificity%	PPV	NPV
<0.02 ng/ml on arrival	87%	62.79% 51.70%-72.98	94.07% 88%-95.81	60.00% 51.23-68.17	94.69% 93.13-95.92
<0.08 ng/ml on arrival	94%	43.02% 32.39% 54.15	99.18% 98.09%-99.73	88.10% 74.94%-94.82	92.47% 91.09%-93.66
<0.02 ng/ml on arrival & 3 hours post arrival	76.4%	83.72% 74.2-90.80	84.92% 81.83% to 87.67%	43.90% 38.8 -49.12	97.37% 95.8-98.36
<0.08 ng/ml on arrival & 3 hours	90%	65.12% 54.08%-75.08%	98.69% 97.43%-99.43%	87.50% 77.56%-93.41%	95.25% 93.76%-96.40%
<0.01 ng/ml on arrival & 3 hours	47%	94.19% 86.95%-98.09%	53.28 % 49.23%-57.30%	22.13% 20.46%-23.90%	98.48 % 96.51%-99.35%

PPV: Positive predictive value, NPV: Negative predictive value, Rule-out strategy, POC cTn <0.02 ng/ml Lower limit of detection cut-off, 0.08 ng/ml the conventional 99th percentile cut-off, POC cTn <0.01 ng/ml: the functional sensitivities for the cTnI method

5.4.6 Diagnostic Accuracy of the Functional sensitivity of i-STAT POC on arrival and 3 hours post arrival with ischemic ECG

In 702 patients with complete data of ECG and the maximum i-STAT troponin result of chest pain patients presented to ED, we have evaluated the diagnostic accuracy of i-STAT troponin results at the LoD .02 ng/ml at presentation and three hours post presentation to (EDs) in combination with the emergency physician's assessment of ECG for ruling out AMI within 30 days. We observed that Troponin concentrations were below the lower limit of detection (0.02 ng/ml) in 582 (75%) out of 786 patients. However, this cut-off, together with an ECG of acute ischemia, would allow up to 45.7% of patients among 702 patients to be discharged with a sensitivity of 94.19% (95% CI, 86.95% -98.09%) and an NPV 98.43 % (95% CI 96.38% -99.32%) and specificity of 51.57 % (95%CI,47.51%-55.61%) and a PPV 21.60% (95% CI

20.00%-23.30%). None of the five (7%) patients with false-negative results died within 30 days of presentation. The results are summarised in Table 5.14 and 5.15.

Table 5-14. Two x two table and calculation of negative and positive predictive value as well as sensitivity and specificity for the rule-out and rule- in myocardial infarction on arrival and three hours later of patient presentation with the functional sensitivity

		AMI	No AMI	Total
Maximum i-STAT result and ECG interpretation Strategy	Test +ve	TP 81	FP 295	376
	Test -ve	FN 5	TN 321	326
Total		86	616	702

Maximum i-STAT result: The highest i-STAT troponin recorded, ECG interpretation: ECG shows acute ischemia

Table 5-15. Rule-out strategy, POC cTnat the functional sensitivity of the assay <0.1 ng/ml on arrival & 3 hours

Diagnostic test performance measure	Value%	95% CI	Percentage 'ruled out' (Total FN+TN)/100
Sensitivity	94.19%	86.95% to 98.09%	326/702×100=46%
Specificity	52.11	48.08% to 56.12%	
PPV	21.54%	19.94% to 23.24%	
NPV	98.47 %	96.47% to 99.34%	

5.4.7 Diagnosing AMI

The primary outcome of the study was the diagnosis of AMI, however patients were considered to have AMI if troponin results were above the 99th percentile as per the Third Universal Definition and based on absolute delta by calculating the maximum difference between two troponin levels at time 0 and 3 hours after patient arrival in ED (Jaffe, 2013). The reference standard for AMI was serial central laboratory cTn testing over for at least 3 hours (for high sensitivity assays) or at least 6 hours (contemporary assays) according to the protocol of the different sites.

5.5 Discussion

The reliable determination of troponin even at concentrations well below the previously defined 99th percentile values has become possible. Concentrations below the 99th percentile measured with several different assays have been shown to provide robust prognostic information. In this study we determine the diagnostic accuracy of a contemporary POC cTn assay used on arrival and 3 hours later in patients with suspected ACS, at the conventional 99th percentile and novel LoD cut-offs (Parikh *et al.*, 2008). In order to support emergency medicine physicians in the appropriate triage and management of chest pain patients there has been a prompt improvement in testing threshold sensitivity for POC cardiac troponin testing. Several attempts have been made to detect cardiac troponin alone or in addition to another cardiac marker panel with lower detection thresholds and more accurate detection resulting in a numerous and different POCT research studies (Peacock *et al.*, 2016; Lee-Lewandrowski *et al.*, 2011b). The main finding of this study is that the i-Stat, Abbott POC assay detected 12% of all patients with AMI in the ED by setting the cut-off set at the LoD of 0.02 ng/ml. In this study we found that the LoD cut-off had significantly greater sensitivity than the 99th percentile (95%CI, 83.72% 74.2-90.80) but lower specificity (95%CI, 43.90% 38.8 -49.12%) than the 99th percentile cut-off. However, the use of an unconventional cut-off set at the LoD of a contemporary POC cTn assay substantially improves sensitivity.

With the current study strategy serial sampling over 3 hours, the probability of a missed AMI diagnosis would have been 2.63% using the LoD cut-off and 4.7 % with the 99th percentile cut-off. This could be used to 'rule out' AMI within 3 hours in almost half of all patients if clinicians and patients are willing to accept a small risk of missed AMI. This findings makes an important support to the use of the LoD as a clinical cut-off in results obtained from POC devices to support the clinical judgment as those assays are not highly sensitive as the laboratory assays.

The findings of the current study also corroborate previous research that highlights the analytical sensitivity of the POC troponin I assay (i-STAT). The

study indicated that more AMI cases are detected by lowering the decision cut-off to half of the 99th percentile troponin concentration on the i-STAT using the 0.04 ng/ml cut-off. In this study Schneider et al evaluates the analytical sensitivity of POC troponin I assay (i-STAT) and more sensitive troponin assays (Beckman CoulterAccuTnl and Abbott Architect cTnl). However, the true positive rate in the study was improved to 81% and the false negative rate decreased to 6.8% while the false positive rate increased to 7.3% (Schneider *et al.*, 2013). Conversely, to current study were the false negative rate of the LoD .02 ng/ml was 4.6%. Our finding is significantly higher clinical sensitivity. Despite the low clinical sensitivity if emergency physicians rely on i-STAT result only to rule out patients, combining this finding with clinical information would support the diagnostic accuracy of a clinical decision rule in an ED setting.

This is the first study exploring the diagnostic accuracy of i-STAT POC (cTnl) testing for serial blood sample on arrival and 3 hours later in patients with suspected (ACS) at the conventional 99th percentile and novel LoD cut-offs. In this study, the diagnostic sensitivity of the i-STAT POC assay at the LoD at patient presentation was 62.79%. Recently, a study by Sardi, et al, supports lowering the cut-off value of the i-STAT POC troponin values from the 0.08 as suggested by the manufacturer to 0.035 to be in line with the results of the gold standard troponin (Sardi *et al.*, 2016). On the other hand, the results of the current study provides evidence supporting the good performance of i-STAT POC testing for ruling out AMI when the 3 hour test is added after patient presentation to ED. With the additional test performed at 3 hours, later the diagnostic sensitivity was improved to 83.72% and 97.37% NPV. 5 % of patients will miss a diagnosis with this strategy.

The finding of the current study is in line with one of the previous study by Krone et al., 2007 who suggested that in patients with a high clinical probability of ACS increase in troponin above the lower limit of detection (LoD) but below the ESC/ACC decision limit recommendation is also related with greater risk of complication) (Krone, 2007).

In the area of high sensitivity assay, several studies had been previously shown that lowering the diagnostic threshold of the assay was associated with a change in clinical practice, with more referrals to cardiac specialists, more coronary angiography, and wider use of evidenced based treatments (*Body et al.*, 2016b; *Bandstein et al.*, 2014). To date, many studies compare conventional central laboratory assays to POC troponin, but insufficient data is available on the comparison of diagnostic accuracy of POC testing with hsTn troponin assay. The diagnostic performance of hsTnT and troponin POC at the 99th percentile and conventional rule in cut offs has been evaluated in a study by Slagman and his group, clinical routine troponin samples from 3432 patients presenting to the ED was tested in both assays. 3.6% of patients had a diagnosis of NSTEMI for the hsTnT assay, 28.4% of all values were at or below the (LOD) as compared to 75.7% of the POC-TnT-values. The diagnostic performance was similar for both assays, the sensitivity at the 99th percentile cut off for hsTnT and POC was 92% and 85% respectively, the NPV at the 99th percentile was similar for both assay 99% (*Slagman et al.*, 2017). This finding is in agreement with results of the previous year by *James et al.* In this study the researcher compared the performance of a POCT cTnI assay in contrast to a central lab cTnT assay. The odds ratio for 30-day death or MI was 1.64 (95% CI: 1.31-2.06) for the POCT assay and 4.29 (CI: 3.02-6.09) for the central lab assay (*James et al.*, 2004). Other results are similar to this finding in a study by *TerAvest et al.*, the researchers compared the analytical and diagnostic performance of the AQT-90 POC TnT assay with central laboratory hsTnT in 261 samples from patients presenting to ED of whom 13% had a final diagnosis of NSTEMI. The authors report a much lower sensitivity of the POC TnT (68% vs 85.48) but a very similar sensitivity for hsTnT (91% vs. 92.74%), and also a lower NPV of the POC TnT (95% vs. 99.37%) (*TerAvest et al.*, 2016). A study by (*Venge et al.*, 2010) showed that the POC cTnI assays are less sensitive for outcome prediction of patients with myocardial injury. The researcher evaluated two different POC assays with a central laboratory assay. The i-Stat (Abbott Diagnostics) and Stratus CS (Siemens Healthcare Diagnostics) as POC assays Access AccuTnI (Beckman Coulter, Fullerton, CA) and Architect cTnI (Abbott Diagnostics) as laboratory assays. However, they found that by implementing the 99th percentiles upper reference limit, the

Access AccuTnI identified 88% and Architect cTnI identified 81% of all patients who died of cardiovascular disease as compared with 50% and 54% for i-Stat and Stratus CS, respectively. The high negative predictive values for the laboratory assays were 97% as compared with 89% to 93% for the POC assays. Negative likelihood ratios were 0.25 (CI 0.15-0.041) and 0.59 to 0.68 (CI 0.47-0.79), respectively. Another study by (Palamalai *et al.*, 2013) evaluated four point of care cardiac troponin I assays to rule in and rule out AMI they found out that the analytical variability that exists between POC cTnI assays determines substantial diagnostic differences for ruling in and ruling out AMI in patients presenting with symptoms suggestive of ACS.

This finding is particularly important to assist the triage system and patient management in the ED environment (Storrow *et al.*, 2008). With the optimal goal for patient satisfaction outcome and improving the health system overall (Thygesen *et al.*, 2010). In the current study we evaluated the diagnostic sensitivity therefore we could improve the true positive detection rate of such assay with an acceptable number of false positive results in a real life population made in EDs. In the current study i-Stat showed comparable performance to the central laboratory high sensitivity assay. This study demonstrates the potential of quantitative of i-Stat, Abbott POC cTn assay measurement in the ED for diagnostic performance of AMI.

When high sensitivity troponin assays were developed in the market this has been raised the emphasis on earlier diagnosis and faster discharge from EDs and the gap between point of care troponin and laboratory might extend. On the other hand, a study reported that POC troponin assays identify the majority of patients with ACS (Rathore *et al.*, 2008). Results that indicate a need for rapid clinicians intervention should be delivered to requesting clinical as a matter of urgency. Reporting troponin result is until now facing challenges in interpretation of results in the ED. For this reason, it is very important for clinicians to understand certain basic characteristics of assays, and also work should be done to improve the reference intervals and establishment of decision thresholds in order to critically interpret the troponin assay. This is essential for the successful implementation of the assay for the use in clinical practice.

Delivering troponin results over an appropriate timescale may provide therapeutic and psychological benefits to patients and to the health system overall from the starting of patient safety to the cost effective for patient care. There is a significant differences in analytical characteristics of troponin assay. Each assay requires an individual assessment protocol to be set between the emergency physician and laboratory specialist. In the meantime, there are a concerns around the lack of analytical sensitivity of POC assays compared to laboratory assay were POC assay have higher level of imprecision and this resulted in lower clinical sensitivity for AMI diagnosis in ED.

This study demonstrates the importance of presenting both sensitivity and NPV to evaluate the diagnostic accuracy of early strategies to exclude AMI. In this study, the Low concentrations at LoD for i-STAT cardiac troponin I determined on presentation and three hours later to the emergency department (ED) showed to have an excellent negative predictive value (NPV) 96.36% for the identification of acute myocardial infarction when combined with ECG. A study similar to our finding has been evaluated the clinical performances of another POC device (MinicarecTnl). The researchers found that, with the addition of the three hours sample the NPV was increased to 99% three hours later instead of 95% for the initial sample only (Venge et al., 2017).

The selection of an appropriate diagnostic threshold requires careful consideration of concentrations seen in the normal and disease free population and those seen in diseased populations. There is always a continuous balance between the sensitivity and specificity of any marker. If the cut-off concentration used is low, the sensitivity is improved at the expense of specificity unless the marker is 100% cardiac specific, and only present in diseased populations.(Henderson and Bhayana, 1995).

However, this allows the use of low cut off concentration that improves the utility of troponin for risk stratification in our main project (BEST Study). In addition, the main advantage of using the i-STAT POC is the ability to meet the turnaround time of a cTn result within 60 min from the time of phlebotomy to the result availability to emergency physician. Recent evidence supports the

use of hs-cTn assays in rule out of MI within 1 hour after admission. Using the laboratory assays often delayed the turnaround time recommended for troponin testing. Since the clinicians depend on fast TAT in order to achieve early diagnosis and treatment of chest pain patients and more importantly to achieve early discharged form ED. Implementation of sufficiently sensitive POC cTnl assay could support earlier discharge by providing a rapid cTnl measurement (Bingisser *et al.*, 2012). A recent meta-analysis by S. Goodacre et al reported that the sensitivity of troponin at patient presentation is about 70-80 % and this is depending on the assay used (Goodacre *et al.*, 2013). The clinical performance of i-STAT POC has been reported in diagnostic evaluation study of four POC cardiac troponin I assays. In this study, researchers compared the diagnostic sensitivity of different POC to a central laboratory cTnl assay for detecting myocardial injury and diagnosing AMI. With Abbott, i-STAT troponin assay plasma was obtained at presentation, 3 hours and 6 hours for 169 patients presenting with symptoms suggestive of (ACS). They find out that there was a significant ($p < .005$) improvement at 3 h and 6 h post presentation for all POC and the sensitivity was varied between the assays. Clinical sensitivity for i-stat at time zero was 32% (13, 57), 68% (43, 87), and 68% (43, 87) at presentation, time three and time 6 hours respectively. All PPVs in the study for all POC tests were $\leq 50\%$ at all time-points (Palamalai *et al.*, 2013).

Previous work has evaluated the diagnostic performance of VITROS(R) Troponin I-ES assay (Ortho-clinical Diagnostics, Rochester, NY) and reported that in patient admission and 6-hours Tnl levels with this device had AMI sensitivities of 69% (95% CI 55%-81%) and 94% (95% CI 84%-99%), respectively. The specificities were 78% (95% CI 73%-82%) and 81% (95% CI 77%-85%) and MI was clinically diagnosed in 52 (13.6%) of patients during initial hospitalisation (Apple *et al.*, 2009). Similar findings of our result have recently been described by Diercks et al (2012) as they reported that 9.6% patients from total of 858 patients were diagnosed with AMI. The sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio over 3 hours were 84.1%, 93.4%, 12.8, and 0.17, respectively when using the Cardio 3 Tnl POC device in ED. There was no significant improvement in diagnostic

accuracy associated with adding 6-hour serial testing to the 3-hour sample and there was no change in diagnostic accuracy between 0 time and 3 hours of testing however (Diercks *et al.*, 2012). Existing data suggest that POC cardiac markers can accelerate decision-making in the ED. Goodacre *et al.* 2011 evaluate the clinical effectiveness of POC cardiac marker panel in patients presenting to the (ED) with suspected AMI the study conclude, that the time to ACS diagnosis is faster when POC testing is performed and the diagnostic at 4 hours was: Odds ratio: 3.81 (3.01 to 4.82, $p < 0.001$)(Goodacre *et al.*, 2011). The first-draw sample for cardiac marker testing is very important. Previous study evaluated POC with old generation troponin assay and reported that only 63% of patients with an MI showed a positive cardiac marker on the first draw specimen using a multi marker platform with an older, qualitative, POCT cardiac marker technology with a specificity of 81 % (Kratz *et al.*, 2002). A similar finding for our study has recently been reported the diagnostic sensitivity of i-STAT POC when first draw of troponin sample at time 0 of patient presented to ED is used. The sensitivity of AMI using i-STAT was 63% and NPV was 95% and 10.8% of AMI identified in the study (Lee-Lewandrowski *et al.*, 2011a). Another study evaluate the diagnostic of POC (Spectral Cardiac STATus) at time 0,4h and 8 hours comparing POC testing to the central Stratus laboratory assay the sensitivity at time 4 was 88.0% (82.4 to 92.1) and NPV was 97.0% (90.8 to 99.2) (Hillis *et al.*, 1999).

In conclusion, the use of an unconventional cut-off set at the LoD of a contemporary POC cTn assay substantially improves sensitivity. However, this could be used to 'rule out' AMI within 3 hours in almost half of all patients if clinicians and patients are willing to accept a small risk of missed AMI. Next chapter will demonstrate the diagnostic accuracy of the T-MACS accelerated diagnostic protocol celebrated with Troponin tested by the (i-STAT).

5.6 Strength of the study

The analytical sensitivity gap between central laboratory testing platforms and POCT assays for cardiac troponin is very important and has limited the adoption of POCT for several hospitals. The strength of our study is to improve the clinical sensitivity of the i-stat POC test with a novel LoD cut-off. The

current study is unique in several ways. (1) this is the first study to evaluate early rule out strategy for Acute Myocardial Infarction using an unconventional cut-off set at the LoD of a contemporary POC cTn assay (i-STAT) (the optimal 'delta' troponin (serial measurement) for the assay and ECG interpretation., (2) this study has evaluated the diagnostic accuracy of the POC i-STAT troponin assay in combination with the emergency physician's assessment of an ECG for ruling out AMI. To our knowledge, no previous study so far has evaluated this combination. (3) the findings of this study will increase the evidence-based literature for implementing the i-STAT cTnI POC sitting at the LoD cut-off system along ESC/ACC guidelines. Finally,(4) this study was focussed on the clinical performance of the respective assay in terms of their ability to identify a level of cTnI that would support a diagnosis of MI (i.e. above the L cut-off).

5.7 Limitation and strength of the study

One of the limitation of this study is the lack of consideration for the coefficient of variation. It is agreed that a diagnostic tool should be able to establish a cut-off score as long as the coefficient of variation (mean/standard deviation) at this point is less than 10%.(Roffi *et al.*, 2016). In the current study, a cut-off of 0.02 ng/ml was studied, which does not satisfy these criteria. It has been reported that, for this assay, the total imprecision (CV) of 10% and 20% were seen at 0.09 and 0.07 respectively (Apple *et al.*, 2004).

**Chapter 6 : Multicentre, prospective validation of the Troponin-only
Manchester Acute Coronary Syndromes decision aid using a single
point of care troponin test in the Emergency Department**

6.1. Background

Acute chest pain is a very common presentation at emergency departments worldwide. Almost 700,000 patients present to the ED per year in England and Wales, which equates to approximately 25% of emergency medical admissions (Goodacre *et al.*, 2005). However, reports suggest that as little as 10% of patients are diagnosed with AMI (Than *et al.*, 2014b) and 15% are diagnosed with ACS (Pollack *et al.*, 2006). From the clinical point of view, risk assessment, referral of patients with a suspected ACS is highly important for patient health and survival. The prompt evaluation of such patients is important, as prolonged assessment contributes to high workload, ED overcrowding, occupying bed spaces, which is placing an additional burden on health care systems resulting in adverse patient outcomes, and increased mortality (Bernstein *et al.*, 2009). Distinguishing whether a patient presenting with chest pain has an acute coronary syndrome or a non-cardiac problem is difficult (Carlton *et al.*, 2015a). However, Emergency physicians must rapidly identify and treat patients with chest pain whilst also avoiding unnecessary investigation and admission for the group of patients who can be safely discharged. It has been reported that 6% of patients discharged from a UK emergency department have subsequently been proven to have prognostically significant myocardial damage (Collinson *et al.*, 2000). Another study found the rate of missed diagnosis in the ED to be 2% (Suter, 2007). Most importantly, the majority of hospital admissions for chest pain could be avoided with improved diagnostic technology such as POCT. Currently the lack of sensitivity and NPV of existing diagnostic technology at the time of patient arrival in hospital leads to unnecessary hospital admissions and contributes to overcrowding of ED. On the other hand, there is a challenge to maintain a reasonable specificity and PPV. However, there are excessive and developing strategies that would permit early exclusion of acute coronary syndromes (ACS) in ED. The availability of accelerated diagnostic protocol in ED could avoid unnecessary hospital admissions and facilitate patient triage. The risk assessment should be performed as soon as a clinical diagnosis of ACS is made according to The National Institute of Health and Clinical Excellence (NICE) guidelines; also, it must be based upon rapidly available clinical and

laboratory information (Cooper *et al.*, 2010). Additionally, it is recommended that the accelerated diagnostic protocol selected is user-friendly, suitably validated, and conformable between observers and users. Numerous risk stratification scores are available and are based on clinical history, ECG findings and cardiac markers (Antman *et al.*, 2000; Granger *et al.*, 2003; Body *et al.*, 2014; Six *et al.*, 2012). One of the most commonly used is the (TIMI) risk score. This score was designed, established and validated in cohorts of patients with ACS (Antman *et al.*, 1999). Previously, it has been reported that over half of elderly patients who do not have AMI have elevated troponin levels on arrival when high sensitivity troponin assay is applied (Reiter *et al.*, 2011). Decision rules combining clinical features, ECG findings and novel biomarkers have been derived to improve risk stratification for patients with ACS have been developed recently. See section 1.5.

The Manchester Acute Coronary Syndrome (MACS) rule (Body *et al.*, 2014c) and Troponin- only MACS (T-MACS) rule (Body *et al.*, 2016a) are clinical decision aids which prospectively validated rule out and risk stratification strategy for ACS based on a single blood test on arrival in patients presenting with suspected cardiac chest pain to the ED. The computer based MACS model involves a combination of high-sensitivity troponin T (hs-TnT), (h-FABP), ECG findings and clinical data as described earlier). Both rules are designed to give the probability that a patient will undergo a major adverse event. See section 1.4.3.5.

In recent years, the number and percentage of patients spending over four hours in A&E departments has increased. It has been reported recently in the latest Accident and Emergency NHS Statistics that in 2016, more than 16.2% of patients were classified major and spent over 4 hours in A&E. This is highest percentage for over a decade as reported. In 2016 compared to 2015 the number of patient attendances increased by 5.2%, which is equivalent to an average of 3,200 more people attending A&E each day in England (Baker, 2017). The number of attendances is raised each year and this will affect the health outcome if the patient is not treated as soon as possible. However, with the long turnaround time of central laboratory tests and the chance to rule in patients without AMI and having high troponin result tested by high sensitivity

assay, the implementation of POC troponin in combination with a clinical decision rule could help in 'rule in' and 'rule out' ACS in the ED setting. Nevertheless, the evaluation of serial troponin testing by POC incorporation with a clinical decision rule is not yet available. Recently, there has been much interest in POCT, as it has the benefit over central laboratory-based assays of reducing the time and delivering results faster to clinicians as mentioned earlier. Moreover, it is capable of reducing ED overcrowding, speeding up patient management process, reducing hospital admissions, assisting in appropriate treatment plan and finally supporting and extending laboratory testing within the hospital. Several type of POC devices are available in the market Figure 6.1. However, implementing POC device/test in a busy environment such as ED is challenging, as the analytical characteristics of these assays are significantly different from central laboratory assays. Hence, diagnostic studies and research clinical trials are needed to evaluate the effectiveness of such devices in patient's outcome and risk stratification in emergency setting.

In the United Kingdom, the National Health Service (NHS) has a government target of four hours within which minimum of 95% patients attending ED should be diagnosed, treated and then admitted or discharged. However, the target for 95% of all attendees to be discharged, admitted or transferred within 4 hours has not been met in the monthly data since July 2015 as reported in latest statistics report from NHS (Vile *et al.*, 2017).

All patients with acute chest pain who are assessed as having medium or high risk of a (MI) should be given high sensitivity troponin blood tests, following the guidance from National Institute for Health and Care Excellence (NICE) advises. (NICE) recommends that two blood tests for high sensitivity cardiac troponin should be taken three hours apart. However, with the turnaround time of a central laboratory test, this would mean all patients are admitted to hospital. This concern raised the need to validate the T-MACS rule with contemporary cardiac troponin assays such as (i-STAT) where this assay do not meet criteria for being labelled as 'high sensitivity' assay.

6.2. Aim and objectives

The aim of this study was to prospectively validate T-MACS with a contemporary POC cTn assay (i-Stat, Abbott Point of Care, New Jersey) in order to investigate the clinical diagnostic accuracy of the i-Stat device to rule out AMI in ED.



Figure 6-1. A variety of POCT platforms

(A) Cardiac Reader, Roche Diagnostics; (B) Stratus CS, Siemens Healthcare; (C) Triage, Alere; (D) Cobas h232, Roche Diagnostics; (E) iSTAT, Abbott Laboratories; (F) Meritas, Trinity Biotech; (G) LABGE01B10, Samsung; (H) PATHFAST, Mitsubishi Chemical Europe and (I) AQT90, Radiometer. Picture adapted from (Gaze, 2016).

6.3. Methods

6.3.1. Participants

During the study time frame of February 2015 to March 2017 there were 1,613 patients enrolled. Of this cohort, some patients were excluded for missing i-

Stat on arrival, and the patients who did not have an ECG recorded were also excluded. This left 634 patients in the final group for analysis consisting of 457 men (62.3%) and 276 women (37.7%). The mean age was 58 years (standard deviation 16). In this study, we included patients who were presented to the ED with a primary complaint of chest pain and the emergency physicians suspected might be cardiac in nature and warranted further investigation for a possible ACS. Samples were analysed by POC (i-Stat assay (Abbott Point of Care, New Jersey) set in the ED of 14 hospitals around the UK. Inclusion and exclusion criteria were described in detail earlier section 2.1.1.3.1. All participants provided written informed consent.

6.3.2. Laboratory analyses and data collection

Blood samples drawn on arrival were analysed for cTnI using the POC i-Stat assay (Abbott Point of Care, New Jersey, 99th percentile 0.08 ng/mL (µg/L), LoD 0.02 ng/mL, 0.10 ng/mL, and functional sensitivity at 10% CV). All patients have undergone serial central laboratory cTn testing over at least 3 hours (if a high sensitivity assay was in use) or at least 6 hours (for contemporary assays) for reference standard following the recommended guidelines. The initial ECG findings and systolic blood pressure were recorded at the time of patient arrival in the ED. ECG in the study were interpreted by the treating clinicians.

6.3.3. Application of the T-MACS decision aid with a POC cTn test

The T-MACS decision aid uses a formula to calculate the probability (p) that a patient has ACS (defined as a diagnosis of AMI relating to the patient's initial presentation or the occurrence of a MACE within 30 days). In this study, the original T-MACS formula was applied using the POC i-Stat assay (reported in ng/L). The T-MACS rule estimates the probability (p) of acute coronary syndromes as follows:

$1/(1+\exp(-4.77+1.71E+0.85A+0.61R+1.42V+2.06S+1.21B+0.089T))$, where E is evidence of acute ECG ischaemia, A is worsening angina, R is pain radiation to the right arm or shoulder, V is pain associated with vomiting, S is sweating observed, B is systolic blood pressure <100mmHg and T is cardiac troponin concentration (ng/L) on arrival in the ED.

Consistent with the previous approach, patients with cTnI concentrations below the reportable range of the assay (10 ng/L) were entered as 9ng/L. The calculation in real clinical practice would be computer-based. The predictors in the T-MACS measurement scale are shown in (Table 6.1). For dichotomous variables, a value of '1' is entered for 'yes' and '0' for 'no'. The estimated probability of each group is described in (Table 6.2)

Table 6-1. Predictors in the Manchester Acute Coronary Syndromes (MACS) model Variable Measurement scale

Variable	Measurement Scale
Acute ECG ischaemia (treating clinician's interpretation)	Dichotomous
Worsening (or crescendo) angina	Dichotomous
Pain radiating to the right arm or shoulder	Dichotomous
Vomiting in association with the presenting	Dichotomous
Sweating observed by the treating clinician	Dichotomous
Systolic Blood Pressure <100 mm Hg on arrival	Dichotomous
Admission cTn by i-STAT (ng/L)	Continuous

6.3.4. Statistical analysis

The diagnostic accuracy of T-MACS was evaluated by calculating sensitivity, specificity, PPV and NPVs, positive and negative likelihood ratios. All statistical analyses were performed using either SPSS version 22.0 (IBM Software, USA) or MedCalc online calculator version. The model classified patients into four distinct risk groups based on their calculated risk probability according to the cut offs applied in the derivation of the original TMACS rule. The four risk groups with associated suggestion for patient disposition are presented in Table 6.2. (ROC) curves were constructed to assess the diagnostic accuracy and the corresponding sensitivity and specificity of the T-MACS score estimate the probability of risk group.

Table 6-2. T-MACS Groups risk probability

Risk group defined by T-MACS	Probability threshold in the T-MACS Rule	Interpretation
Very low risk	<2%	ACS ruled out consider discharge
Low risk	Between 2% and 4.9%	Consider serial troponin in ED e.g. 3h troponin then consider discharge if normal
Moderate risk	Between 5% and 94.9%	Serial troponin in general ward and consider stress testing and/or CT coronary angiography thereafter.
High risk	>95%	ACS ruled in, refer to cardiology for treatment

6.3.5. Follow-up

All patients were underwent reference standard troponin testing in accordance with contemporary national and international guidance. Acceptable protocols for reference standard troponin testing include:

- If a contemporary (not high sensitivity) troponin assay is used: Laboratory-based troponin testing on arrival and either 6 hours after arrival or 10 to 12 hours after the onset of peak symptoms (Thygesen et al., 2010).
- If a high sensitivity troponin assay is used: Laboratory-based troponin testing on arrival and either 3 hours after arrival or 10 to 12 hours after the onset of peak symptoms (DG15, 2014; Apple *et al.*, 2002).

A high sensitivity troponin assay is defined as an assay that can detect troponin concentrations in at least 50% of apparently healthy individuals with a CV <10% at the 99th percentile cut-off (Apple *et al.*, 2012a).

All participants were followed up by telephone, email, letter, home visit or in clinic after 30 days. If the patients were un-contactable, the research nurses contacted the general practitioner for important information needed for results.

6.3.5. Outcome

The primary outcome of this study is rapid diagnosis of acute myocardial infarction. Outcomes was adjudicated by two independent investigators with reference to relevant clinical information but blinded to the results of research investigations. Discrepancies was resolved by consultation with a third independent investigator. AMI was defined according to the Third Universal

Definition of AMI (Jaffe, 2013) however, by the inclusion criteria, all patients has symptoms and signs consistent with myocardial ischaemia. Therefore, patients who deemed to have met this outcome if they develop a rise and/or fall of troponin to above the 99th percentile. At the time of writing this thesis and pending further evidence and international guidance, a significant rise and/or fall of troponin was considered to be >9.2ng/L for high sensitivity troponin T (Mueller *et al.*, 2012) and at least 20% for all other assays.

6.4. Results

634 patients were included in this study between February 2015 and March 2017 all of whom completed follow-up. Table 6.3 illustrate baseline characteristics in patients among the study. In this study 76 patients 12% of patients had AMI, proportion of patients with AMI in each of the T-MACS risk groups are illustrated in Figure 6.2 and Table 6.4. In the current study, with a single i-STAT cardiac troponin I (cTnI) assay threshold of <0.10 ng/mL, The T-MACS rule successfully stratified patients according to their risk of AMI, T-MACS would have 'ruled out' as per the Table 6.5 and Table 6.6 it is 41.8%% (n=265) with a sensitivity of 97.4% (90.8 - 99.7%) and NPV of 99.3% (97.1 – 99.8%). On the other hand, the T-MACS would have 'ruled in' as per the Table 6.7 and Table 6.8 it is 7% (n=44) patients with a specificity of 93.56% (91.27% to 95.40%) and a PPV of 50.00% (42.08% to 57.92%). In total 42% of patients would have been classified as 'very low risk' by the T-MACS rule (with a recommendation that they could be immediately discharged) with two (2.3%) missed AMIs. Two AMIs were missed based on serial laboratory cTn concentrations. (CRF) form were checked and hostiles were contacted for follow up. One of these two patients had hs-cTnT concentrations of 6ng/L and 16ng/L on arrival and at 3 hours respectively. The patient underwent coronary angiography, which showed diseased coronary artery bypass grafts that was not felt to require coronary intervention. The other patient had hs-cTnT concentrations of 15ng/L and 35ng/L, was given a clinical diagnosis of AMI and underwent percutaneous coronary intervention eight days after initial admission .T-MACS probability calculated for the risk groups has an AUC of 0.883 (95% CI. 84 to .92) showing a very good discrimination for risk stratification patients in emergency department. See Figure 6.3

Table 6-3. Baseline characteristics of included patients

Variable	Total
Age in years, mean (SD)	55 (15.5)
Men (%)	62.3
<u>MAIN SITE OF PAIN</u>	
Central chest	64.5%
Left anterior chest	25.8%
Left lateral chest	6.6%
Right anterior chest	3.5%
Epigastrium	5.7%
Back	4.9%
Arms/shoulders	9%
Jaw/neck/throat	7.8%
<u>PAIN RADIATION</u>	26.8%
Left arm	
Left shoulder	12.5%
Right arm	5.6%
Right shoulder	4.3%
Throat or jaw	11.8%
Neck	9.9%
Back	8.7%
Left chest	5.6%
Right chest	2.9%
Legs	2%
<u>ASSOCIATED SYMPTOMS</u>	22.1%
Nausea	
Vomiting	5.2%
Dyspnoea	24%
Light-headedness	19.5%
Vertigo	3%
Sweating	30%
<u>PAST MEDICAL HISTORY</u>	
Previous myocardial infarction (%)	22.9%
Previous angina (%)	26%
Previous PCI/stent	18.9%
Previous CABG	6%
Hyperlipidaemia (%)	35%
Hypertension (%)	45.3%
Type 2 Diabetes mellitus (%)	16.4%
<u>RELEVANT SOCIAL HISTORY</u>	
Current smoking	20%
Alcohol use	36%
<u>ECG interpretation:</u>	7%
Acute ischaemia	
ST elevation (STEMI)	5%
LBBB	5%
ST depression	10.3%
Abnormal T inversion	7.7%

Table 6-4. Proportion of patients with AMI in each of the T-MACS risk groups total number of patients including in the analysis 634

Risk groups	Number (%) with AMI	Number (%) with no AMI	Total number (%) in risk group
High risk group	TP 38(86.4%)	FP 6 (13.6%)	44 (7%)
Moderate risk	20 (13.5%)	128 (86.5%)	148 (23%)
Low risk	16 (9%)	161 (90 %)	177 (28%)
Very low risk group	FN 2 (.8%)	TN 263 (99%)	265 (42%)
Total	76 (12%)	558 (88%)	634

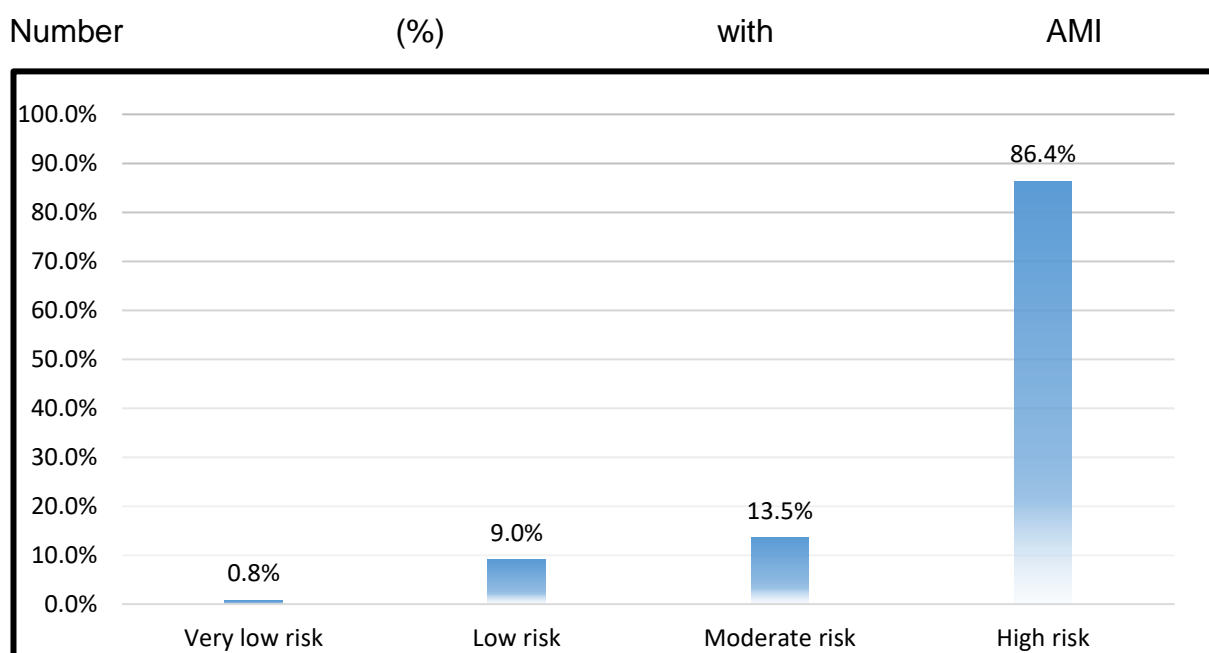


Figure 6-2. Proportion of patients with AMI in each of the T-MACS risk groups

Performance of the (Troponin-only Manchester Acute Coronary syndromes (T-MACS) in the patient population (n= 634). The suggested disposition for each group is as follows: (Very low risk .8%) were patients who were discharged immediately. (Estimated probability of AMI is <2%), (low risk 9.0%) were the patients who were admitted to low dependency environment, such as emergency department observation unit. (Estimated probability of AMI is between 2% and 4.9%), (Moderate risk 13.5%) were admitted to the acute ward, such as medical admissions unit (estimated probability of AMI is between 5% and 94.9%), (High risk 86.4%) were admitted to coronary care unit or high dependency environment (estimated probability of AMI is \geq 95%). AMI: acute myocardial infarction.

Table 6-5. Prevalence of AMI stratified by T-MACS risk group

T-MACS		AMI	No AMI	Total
Patients group	All other risk groups	TP 74	FP 295	369
	Very low risk group	FN 2	TN 263	265
Total		76	558	634

Table 6-6. Test characteristics of T-MACS (very low risk vs all other risk groups)

Diagnostic accuracy of the rule out strategy	Value%	95% CI	Percentage 'ruled out'
Sensitivity	97.4%	90.8 - 99.7%	265/634 x100 41.8%
Specificity	47.1%	42.9 – 51.4%	
PPV	20.1%	18.7 – 21.5%	
NPV	99.3%	97.1 – 99.8%	

Table 6-7. Two x two table showing the prevalence of AMI stratified by T-MACS risk group (high risk vs all other risk groups)

Sensitivity and specificity for the rule-out and rule- in myocardial infarction by T-MACS for high risk group only are also reported

T-MACS	AMI	No AMI	Total
High risk group	TP 38	FP 38	44
All other risk groups	FN 6	TN 552	558
Total	44	590	634

AMI Formula: AMI was defined according to the Third Universal Definition of AMI

TP: True positive, FP: False positive, FN: False negative, TN: True negative.

Table 6-8. Significance of diagnostic Rule-out strategy of T-MACS for high-risk group only

Diagnostic accuracy of the rule out strategy	Value%	95% CI	Percentage 'ruled in'
Sensitivity	86.36%	72.65% to 94.83%	44/634x100 6.9%
Specificity	93.56%	91.27% to 95.40%	
PPV	50.00%	42.08% to 57.92%	
NPV	97.77 %	95.42% to 98.93%	

6.11. ROC curve for T-MACS probability

T-MACS probability calculated for the risk groups has an AUC of 0.883(95% CI. 84 to .92) showing a very good discriminator for risk stratification patients in emergency department see Figure 6.3.

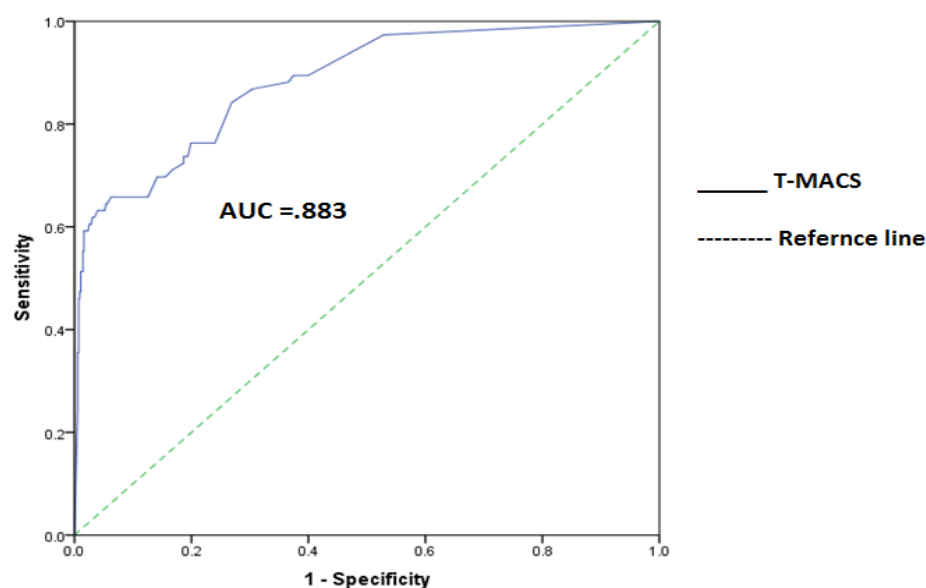


Figure 6-3.

Receiver operating characteristic curves showing the overall accuracy of T-MACS Probability for risk stratification of chest pain patients in ED sitting which considered to 'rule out' strategy in the current study.

6.5. Discussion

The T-MACS rule has been validated based on previous findings that the original T-MACS rule can effectively 'rule out' and 'rule in' acute coronary syndromes (ACS) following a single blood test and the calculation score is able to stratify the risk of adverse events within 30 days in ED patients with suspected cardiac chest pain. POC troponin tests are less sensitive than hs-Tn laboratory assays. However, integrating the POC result into clinical algorithm and delivering rapid result helps in facilitating early decision and delivering rapid results. In the present study, by setting the cut off levels of POC at 0.10 ng/mL and functional sensitivity at 10% CV, the T-MACS rule has successfully 'ruled out' > 41.8% of patients with suspected cardiac chest pain following a single blood test, with a sensitivity of 97.4% (90.8 - 99.7%) and NPV of 99.3% (97.1 – 99.8%), in a very short turnaround time of 5-10 minutes. On the other hand T-MACS has risk stratified the patients who were at high risk to have AMI with a specificity of 93.56% (91.27% to 95.40%) and a PPV of 50.00% (42.08% to 57.92%). Setting a POC assay at low threshold levels could help in several ways, it helps in taking clinical decision in a shorter time, also helps in safe discharge of more patients and finally reduces length of stay (Ryan *et al.*, 2009). While on the other hand, prolonged diagnosis leads to

overcrowding of emergency department, increased cost of health care and resources used (Pines *et al.*, 2009; Thokala *et al.*, 2012). To our knowledge, this was the first study to date that evaluated POC test in combination with the medical history of the patients, ECG interpretation and clinical information in order to exclude AMI in a busy environment setting. In this study population, more than 40% of patients were identified as very low risk group to rule out strategy. Despite the fact that the assay used in T-MACS is less sensitive than hs-Tn laboratory, a threshold of 10 ng/ml for 'ruling out' ACS allowed 41.8% of patients to be discharged with only <2% of ACS cases were undetected. Several clinical decision rules have been derived based on flow of patients in emergency department; however, these strategies were aimed to reduce unnecessary hospital admissions for chest pain patients. Latest studies have confirmed that very low troponin concentrations at patient arrival can be used to risk stratify patients (Body *et al.*, 2015c; Bandstein *et al.*, 2014). Numerous studies of (ADP) using contemporary sensitive cTn assays suggest that early serial sampling time for a period of 60-120 min, in addition to use of either absolute or relative cTn concentration might facilitate safe early discharge of low-risk patients with chest pain. A large multicenter ASPECT study (Asia-Pacific Evaluation) used POCT biomarkers (CKMB, Myoglobin) as well as 3rd generation troponin (Than *et al.*, 2011a). This study has shown a diagnostic sensitivity of 82.9% (95% CI 79-86.2) and NPV of 96.1% (95% CI 95-96.9) to rule out patients and specificity of 56% (95% CI 54.3-57.7) and PPV 20.1% (95%CI 18.2-22.0) to rule in patients. However, the study has shown lack of cost effectiveness using the multimarket POC panel). Few years later, the researchers enrolled the same patient population (of ASPECT) for another study trial (ADAPT), the Accelerated Diagnostic Protocol to Assess Patients with symptoms of chest pain. However, in this study, the researchers used troponin only as a single biomarker in the protocol and their strategy was successful to identify 20% of patients at low risk (392/1975). The ADP protocol had a sensitivity of 99.7% (95% CI 98.1-99.9%) and NPV of 99.7% (95% CI 98.6-100%), specificity of 23.4% (95% CI 21.4-25.4%) and positive predictive value of 19% (95% CI 17.2-21%). However, in this study the researchers used TIMI score which has debateable application to acute ED population since it was originally developed for use in patients with unstable angina or non-ST-

elevation myocardial infarction (Hess and Jaffe, 2012). POCT multi- marker strategy was evaluated in ED setting by some authors. However, to test the advantages of POC in emergency setting especially for turnaround time, other authors (McCord *et al.*, 2001) have evaluated the effectiveness of an accelerated 90 min protocol for diagnosis of AMI in ED setting using serial multi-marker POCT (cTn, CK-MB and myoglobin). In this study all chest pain patients were rapidly diagnosed within 90 min and the admission rate to the coronary care unit was decreased by 40%). Another study by Goodacre *et al* demonstrated the serial evaluation of cardiac marker over 90 minutes in ED setting. The researchers found out that, the implementation of POC could lead to an increase in the proportion of patients who are successfully discharged from the ED by 63% and with high-risk patients, the percentage increases to 83%. On the other hand, there were a total of 120 patients (11.6%) who were successfully diagnosed and treated within 4 hours of arrival to ED. This pilot work suggests that POC cardiac markers can facilitate discharge of low-risk patients from the ED without a significant affecting rate in patient attendance and without affecting the 4-hour waiting target setting by NHS (Goodacre *et al.*, 2011). There is also an alternative way to rule out chest pain patients by evaluating the History, Electrocardiogram, Age, Risk Factors, and Troponin (HEART) score or triage rule-out using high-sensitivity Troponin protocol. It has been addressed that this score can be an alternative way to safely discharge chest pain patients presenting to ED (Leite *et al.*, 2015; Backus *et al.*, 2010; Six *et al.*, 2013). However, the latest systematic review which evaluated the diagnostic accuracy of HEART for early rule out of acute coronary syndromes in ED has been reported to have lower sensitivity of 96.7% (95% CI 94.0-98.2%) and specificity of 47% (95% CI 41.0-53.5%) when compared with current study (Van and Body, 2017). More importantly, that none of these protocols were derived in the area of point of care. Another systematic review on latest predictive tools for the assessment of patients with chest pain has been recently reported which included six different studies out of 19126 initially identified references in emergency setting. The main findings of the study were AMI and angina. One of the study used the probability of not having (CAD) by using certain predictors which were based on age, gender, clinical characteristics of chest pain, any past medical history of cardiovascular

disease and any abnormalities in ECG as an outcome (Ayerbe *et al.*, 2016). A study by Sanches *et al.* presented a tool to triage patients in ED and identified patients at low risk who do not need admission in chest pain unit. Researchers in this observational study found out that patients who do not have any of these five variables could be safely ruled out that they didn't have ACS. The variables included in this tool were- age of over 40 years diabetes, past history of ACS and crushing chest pain (Lopez *et al.*, 2011). This tool was later validated in 4231 patients and again 100% patients (n=213) without any of the five variables were confirmed not to have CAD. However, the usage of this protocol is limited due to its low specificity.

In recent years, there is growing interest supporting the single test LoD rule out strategy. However, despite the advantages and disadvantages of these strategies, T-MACS rule have has the advantage of facilitating rapid diagnosis for chest pain patients in ED and providing useful pre-test probability result to emergency physician. The rule produces nearly 97.37% diagnostic sensitivity for detection of acute myocardial infarction and 99.16 % NPV. Current study describe a clinical decision rule utilising low cardiac troponin concentrations with a cut-off setting at lowest reportable range to risk stratify patients. No previous research has evaluated such approaches within a protocol to rule in/ rule out chest pain patients. A previous study evaluated similar cut-off (0.010 µg/L at 99th percentile were (0.02 ng/ml) for different POC assay. However the researchers found that this cut-off was helpful in stratify patients mainly for the AMI patients as compared to the central laboratory hs-cTnT assay (Dupuy *et al.*, 2017).

This study aims to address the effectiveness of T-MACS using the POC test. From analytical point of view, in the current study we applied the LoD of troponin test in the T-MACS rule. Since the coefficient of variation (CV) of less than 10% was considered most ideal. The CV of 10% and 20% were seen at 0.09 and 0.07 ng/ml, respectively as published in previous work by Apple *et al.* 2004. However, in their work, the i-STAT POC cTnI assay was quite close to meeting the European Society of Cardiology (ESC) and The American College of Cardiology (ACC) guidelines and criteria for 10% imprecision at the 99th percentile. Previous protocols were limited by low specificity and Negative

predictive value. However, this study strongly recommend the Implementation of this decision rule in emergency setting as such a protocol has the potential to improve the efficiency and safety of early rule-out/rule in patients with suspected acute coronary syndrome. Furthermore, a single test of Troponin by POC has the potential to improve patient outcome by delivering quick results that leads to faster therapeutic interventions, diagnosis with minimal inconvenience to patients and no requirement for serial blood sampling to risk stratified patients. Nevertheless, for those patients who were classified as 'low risk' and 'moderate risk', serial sampling for over 3 hrs is required for further investigation as these patients will remain in 'observation zone'). In the current study, two patients were classified "very low risk" by T-MACS and had AML. Lately, a clinical survey of the acceptable risk of MACE event in chest pain patients after patient discharge from ED had reported that <1% of acute MI was missed by emergency physicians in the ED with risk scores including two negative troponins (Than *et al.*, 2013). For those patients who are in 'very low risk' group, a shared decision making might be helpful as this approach is based on several studies that suggested when low-risk patients are appropriately informed of their risk for ACS and are shared in decision-making procedure, approximately 20% lower rate of observation unit admission is promising (Hess *et al.*, 2012b; Rising *et al.*, 2017; Erik P. Hess *et al.*, 2016). See table 6.9 for the comparison of the T-MACS with the current protocol.

Table 6-9. Comparison of the T-MACS with the current protocol

The test characteristics of the 3 ADPs with the best performance (with 95% CI) were as follows:

Study	Sensitivity	NPV	Specificity	PPV
(Christenson <i>et al.</i> , 2006)	99.4% (98.1-99.9)	93.8 % (78.0-98.9)	4.7% (3.9-5.0)	37.2% (36.7-37.4)
(Hess <i>et al.</i> , 2012a)	99.7% (98.3-100)	98.9% (99.3-99.9)	14.1% (13.3-14.3)	39.7% (39.2-39.8)
(Than <i>et al.</i> , 2011a)	99.2 % (97.5-99.8)	98.1 % (94.1-99.5)	23.8 % (22.9-24.2)	42.5 % (41.8-42.7)
Present Study (Rule out strategy of T-MACS for low risk group)	97.37% (90.82-99.68)	99.25% (97.10-99.81)	99.25% (97.30-99.91)	97.37% (90.29-99.33)

6.6. Conclusions

Using information available at the time of patient arrival to ED and results from a single blood test by the i-STAT cardiac troponin I (cTnI) assay, the (Troponin-only Manchester Acute Coronary syndromes (T-MACS), could enable nearly half of the chest pain patients to be immediately discharged. This rule also classified patients to high-risk patients, moderate risk patients, and low-risk patients in addition to very low-risk patients. Additionally this classification according to patient risk probability will support emergency physicians to discriminate patients according to their risk, which helps to speed up the process of patient triage and transfer patients to target specialist referral faster. Moreover, this rule might reduce the stressful environment of ED where patients arrive in pain, and panicky contributes to the complexity and uncertainty of the decision situation. With a single blood test at the time of arrival in the ED, T-MACS identifies >40% of patients as being at 'very low risk'. These patients have <1% probability of ACS, which may be considered to 'rule out'. Similarly, T-MACS can 'rule in' ACS in approximately 7% of patients with high PPV. Future work should focus on evaluation of the impact of T-MACS when used in practice, particularly when combined with serial troponin sampling over 1–3 hours and in conjunction with shared decision-making. To my knowledge, this is the first successful validation of a single test 'rule out strategy' using a POC cTn assay. Emergency department crowding due to prolonged stays and unnecessary hospital admissions results in bed shortages and this contribute to insufficient resource utilization. Thus, improving the ability to accurately rule out AMI could optimize emergency department triage of chest pain patients. However, the impact of T-MACS role in clinical practice will contribute on resource utilization and holds a great promise on health care system. Finally, this strategy will have significant benefits on healthcare services in NHS by reducing hospital admission rate, improve patients length of stay and support the 4 hours NICE recommendation for patients treatment and management. Nevertheless, analysis of economic and cost effectiveness is needed to evaluate the strategy in real time.

6.7. Strength and limitation of the study

This is the first multi-centre study evaluating troponin by POC assay in emergency department within a clinical decision role with assured protocol standardisation and uniformity procedure between the several sites. This gives strength to the current study by having the possibility of inclusion of a wider range of population group. However, this increases the generalizability of the study. Moreover, The advantages of this computer clinical decision role is that it combines clinical information, ECG findings and novel lower reportable range cut-off for cTn i-STAT and result aims to 'rule in' ACS as well as 'rule out' chest pain patients. This new evidence suggest that a model using POC as the only biomarker gives similar sensitivity and high specificity as a 'rule out' tool from previous finding T-MACS (Body *et al.*, 2016a) . Moreover, the turnaround time of POC device in T-MACS might help to risk stratify the patient and rapidly identify very low-risk patients in order to reduce unnecessary hospital admissions. As this is a prospective study with a follow-up of patients for 30 days, it minimizes the risk of selection bias since the outcome of the patient is not known at the beginning. The TMACS role adds a new exposure for which the beneficial health effect of using POC cTn in emergency department can help to safely stratified chest pain patient with acceptable risk. Moreover, the study added numerous outcomes for the benefit of POC in ED setting. It is important to mention that the 4th universal definition has been published in August 2018, However, the reference standard of this study is the 3rd Universal Definition as that was the definition in force at the time that this work has been taking. Conversely, this study has some limitations that require consideration. There are some quality issues associated with POC assay, as the assay employed in the current study is less analytically sensitive than the assays performed in the central laboratory were the precision of the current POC assay using concentration below the LoD did not meet the recommended guidelines by ESC/ACC. This raised the concerns of laboratory about the precision of the assay at the cut-off level used in the current study. However, this was not reflected in a high false negative rate in this study, suggesting that diagnostic accuracy, as a 'rule out' test was not compromised.

6.8. Future directions

While the results of the current study are promising, the effectiveness of such diagnostic strategy requires testing in a future validation study. However, to assess this rule out strategy, a randomised control trial is required. Also, subsequent research should now evaluate the use of T-MACS in the pre-hospital environment and paramedics as this assay allows for high level of clinical decision management of patient community by supporting the clinical judgments for not to transport the patients and identifying patients who benefit from hospital management. Finally, a future impact analysis would be needed to perform regarding the utility of the T-MACS rule in clinical practice. Our work however, emphasize the importance of developing newer generations of POC cTn assay with greater precision at low concentrations.

References

Abbot. (2013) *CARDIAC TROPONIN I (cTnI)*. Abbot [Online] [Accessed on June 2015] <https://www.pointofcare.abbott/int/en/offerings/support/technical-documentation/cartridge-test-information-sheets>

Abbott. (2015-2016) *The i-STAT system* Abbot. [Online] [Accessed on 15 June] https://www.pointofcare.abbott/us/en/home?utm_source=www.istat.com&utm_medium=email&utm_campaign=www.i-stat.com

Agnello, L., Bivona, G., Novo, G., Scazzone, C., Muratore, R., Levantino, P., Bellia, C., Lo Sasso, B. and Ciaccio, M. (2017) 'Heart-type fatty acid binding protein is a sensitive biomarker for early AMI detection in troponin negative patients: a pilot study.' *Scandinavian Journal of Clinical & Laboratory Investigation*, 77(6) pp. 428-432.

Al-Hadi, H. A. and Fox, K. A. (2009) 'Cardiac markers in the early diagnosis and management of patients with acute coronary syndrome.' *Sultan Qaboos Univ Med J*, 9(3), Dec, pp. 231-246.

Al-Otaiby, M. A., Al-Amri, H. S. and Al-Moghairi, A. M. (2011) 'The clinical significance of cardiac troponins in medical practice.' *J Saudi Heart Assoc*, 23(1), Jan, 2011/01/01, pp. 3-11.

Alley, W. and Mahler, S. A. (2015) 'Clinical decision aids for chest pain in the emergency department: identifying low-risk patients.' *Open Access Emerg Med*, 7 pp. 85-92.

Alpert, J. S., Thygesen, K., Antman, E. and Bassand, J. P. (2000) 'Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction.' *J Am Coll Cardiol*, 36(3), Sep, 2000/09/15, pp. 959-969.

Ambrose, J. A. and Singh, M. (2015) 'Pathophysiology of coronary artery disease leading to acute coronary syndromes.' *F1000Prime Rep*, 7 p. 08.

Ammann, P., Maggiorini, M., Bertel, O., Haenseler, E., Joller-Jemelka, H. I., Oechslin, E., Minder, E. I., Rickli, H. and Fehr, T. (2003) 'Troponin as a risk factor for mortality in critically ill patients without acute coronary syndromes.' *J Am Coll Cardiol*, 41(11), Jun 04, 2003/06/12, pp. 2004-2009.

Amsterdam, E. A., Wenger, N. K., Brindis, R. G., Casey, D. E., Jr., Ganiats, T. G., Holmes, D. R., Jr., Jaffe, A. S., Jneid, H., Kelly, R. F., Kontos, M. C., Levine, G. N., Liebson, P. R., Mukherjee, D., Peterson, E. D., Sabatine, M. S., Smalling, R. W. and Zieman, S. J. (2014) '2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines.' *J Am Coll Cardiol*, 64(24), Dec 23, 2014/09/28, pp. e139-228.

Amundson, B. E. and Apple, F. S. (2015) 'Cardiac troponin assays: a review of quantitative point-of-care devices and their efficacy in the diagnosis of myocardial infarction.' *Clinical Chemistry and Laboratory Medicine*, 53(5), Apr, pp. 665-676.

Anderson, J. L., Adams, C. D., Antman, E. M., Bridges, C. R., Califf, R. M., Casey, D. E., Jr., Chavey, W. E., 2nd, Fesmire, F. M., Hochman, J. S., Levin, T. N., Lincoff, A. M., Peterson, E. D., Theroux, P., Wenger, N. K., Wright, R. S., Smith, S. C., Jr., Jacobs, A. K., Adams, C. D., Anderson, J. L., Antman, E. M., Halperin, J. L., Hunt, S. A., Krumholz, H. M., Kushner, F. G., Lytle, B. W., Nishimura, R., Ornato, J. P., Page, R. L., Riegel, B., American College of, C., American Heart Association Task Force on Practice, G., American College of Emergency, P., Society for Cardiovascular, A., Interventions, Society of Thoracic, S., American Association of, C., Pulmonary, R. and Society for Academic Emergency, M. (2007) 'ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-Elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction) developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine.' *J Am Coll Cardiol*, 50(7), Aug 14, pp. e1-e157.

Antman, E. M., Cohen, M., Bernink, P. J., McCabe, C. H., Horacek, T., Papuchis, G., Mautner, B., Corbalan, R., Radley, D. and Braunwald, E. (2000) 'The TIMI risk score for unstable angina/non-ST elevation MI: A method for prognostication and therapeutic decision making.' *JAMA*, 284(7), Aug 16, pp. 835-842.

Antman, E. M., McCabe, C. H., Gurfinkel, E. P., Turpie, A. G., Bernink, P. J., Salein, D., Bayes De Luna, A., Fox, K., Lablanche, J. M., Radley, D., Premmureur, J. and Braunwald, E. (1999) 'Enoxaparin prevents death and cardiac ischemic events in unstable angina/non-Q-wave myocardial infarction. Results of the thrombolysis in myocardial infarction (TIMI) 11B trial.' *Circulation*, 100(15), Oct 12, 1999/10/12, pp. 1593-1601.

Apple, F. S. (2009) 'A New Season for Cardiac Troponin Assays: It's Time to Keep a Scorecard.' *Clinical Chemistry*, 55(7), Jul, pp. 1303-1306.

Apple, F. S., Wu, A. H. B. and Jaffe, A. S. (2002) 'European Society of Cardiology and American College of Cardiology guidelines for redefinition of myocardial infarction: How to use existing assays clinically and for clinical trials.' *American Heart Journal*, 144(6), Dec, pp. 981-986.

Apple, F. S., Collinson, P. O. and Applicati, I. T. F. C. (2012a) 'Analytical Characteristics of High-Sensitivity Cardiac Troponin Assays.' *Clinical Chemistry*, 58(1), Jan, pp. 54-61.

Apple, F. S., Ler, R. and Murakami, M. M. (2012b) 'Determination of 19 Cardiac Troponin I and T Assay 99th Percentile Values from a Common Presumably Healthy Population.' *Clinical Chemistry*, 58(11), Nov, pp. 1574-1581.

Apple, F. S., Pearce, L. A., Smith, S. W., Kaczmarek, J. M. and Murakami, M. M. (2009) 'Role of Monitoring Changes in Sensitive Cardiac Troponin I Assay Results for Early Diagnosis of Myocardial Infarction and Prediction of Risk of Adverse Events.' *Clinical Chemistry*, 55(5), May, pp. 930-937.

Apple, F. S., Murakami, M. M., Christenson, R. H., Campbell, J. L., Miller, C. J., Hock, K. G. and Scott, M. G. (2004) 'Analytical performance of the i-STAT cardiac troponin I assay.' *Clinica Chimica Acta*, 345(1-2), Jul, pp. 123-127.

Armbruster, D. A. and Pry, T. (2008) 'Limit of Blank, Limit of Detection and Limit of Quantitation.' *The Clinical Biochemist Reviews*, 29(Suppl 1) pp. S49-S52.

Ayerbe, L., González, E., Gallo, V., Coleman, C. L., Wragg, A. and Robson, J. (2016) 'Clinical assessment of patients with chest pain; a systematic review of predictive tools.' *BMC Cardiovascular Disorders*, 16, 01/20

09/03/received

01/15/accepted, p. 18.

Babuin, L. and Jaffe, A. S. (2005) 'Troponin: the biomarker of choice for the detection of cardiac injury.' *CMAJ : Canadian Medical Association Journal*, 173(10) pp. 1191-1202.

Backus, B. E., Six, A. J., Kelder, J. H., Gibler, W. B., Moll, F. L. and Doevendans, P. A. (2011) 'Risk scores for patients with chest pain: evaluation in the emergency department.' *Curr Cardiol Rev*, 7(1), Feb, pp. 2-8.

Backus, B. E., Six, A. J., Kelder, J. C., Mast, T. P., van den Akker, F., Mast, E. G., Monnink, S. H., van Tooren, R. M. and Doevendans, P. A. (2010) 'Chest pain in the emergency room: a multicenter validation of the HEART Score.' *Crit Pathw Cardiol*, 9(3), Sep, 2010/08/31, pp. 164-169.

Backus, B. E., Six, A. J., Kelder, J. C., Bosschaert, M. A. R., Mast, E. G., Mosterd, A., Veldkamp, R. F., Wardeh, A. J., Tio, R., Braam, R., Monnink, S. H. J., van Tooren, R., Mast, T. P., van den Akker, F., Cramer, M. J. M., Poldervaart, J. M., Hoes, A. W. and Doevendans, P. A. (2013) 'A prospective validation of the HEART score for chest pain patients at the emergency department.' *International Journal of Cardiology*, 168(3), 2013/10/03/, pp. 2153-2158.

Baker, C. (2017) *Accident and Emergency Statistics: Demand, Performance and Pressure.*: [Online] [Accessed on SEPTEMBER 14] <https://www.england.nhs.uk/statistics/statistical-work-areas/ae-waiting-times-and-activity/>

Bandstein, N., Ljung, R., Johansson, M. and Holzmann, M. J. (2014) 'Undetectable High-Sensitivity Cardiac Troponin T Level in the Emergency Department and Risk of Myocardial Infarction.' *Journal of the American College of Cardiology*, 63(23), Jun 17, pp. 2569-2577.

Banu, K. Y., Niyazi, O. D., Erdem, C., Afsin, D. H. D., Ozlem, U., Yasemin, C. and Afsin, I. (2014) 'Value of heart-type fatty acid-binding protein (H-FABP) for emergency department patients with suspected acute coronary syndrome.' *African Health Sciences*, 14(3) pp. 757-762.

Bartlett, J. W. and Frost, C. (2008) 'Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables.' *Ultrasound in Obstetrics & Gynecology*, 31(4), Apr, pp. 466-475.

Bathia, D. P., Carless, D. R., Viswanathan, K., Hall, A. S. and Barth, J. H. (2009) 'Serum 99th centile values for two heart-type fatty acid binding protein assays.' *Annals of Clinical Biochemistry*, 46, Nov, pp. 464-467.

Bernstein, S. L., Aronsky, D., Duseja, R., Epstein, S., Handel, D., Hwang, U., McCarthy, M., John McConnell, K., Pines, J. M., Rathlev, N., Schafermeyer, R., Zwemer, F., Schull, M., Asplin, B. R. and Society for Academic Emergency Medicine, E. D. C. T. F. (2009) 'The effect of emergency department crowding on clinically oriented outcomes.' *Acad Emerg Med*, 16(1), Jan, pp. 1-10.

Berridge, B. R., Van Vleet, J. F. and Herman, E. (2013) 'Chapter 46 - Cardiac, Vascular, and Skeletal Muscle Systems A2 - Haschek, Wanda M.' In Rousseaux, C. G. and Wallig, M. A. (eds.) *Haschek and Rousseaux's*

Handbook of Toxicologic Pathology (Third Edition). Boston: Academic Press, pp. 1567-1665.

Bhatnagar, P., Wickramasinghe, K., Wilkins, E. and Townsend, N. (2016) 'Trends in the epidemiology of cardiovascular disease in the UK.' *Heart*, 102(24), Dec 15, pp. 1945-1952.

Bhatnagar, P., Wickramasinghe, K., Williams, J., Rayner, M. and Townsend, N. (2015) 'The epidemiology of cardiovascular disease in the UK 2014.' *Heart*, 101(15), Aug, pp. 1182-1189.

BHF. (2011) 'Modifiable and non-modifiable risk factors

BHF. (2015) *CVD STATISTICS – BHF UK FACTSHEET*. [Online] [Accessed on 17 May] [file:///C:/Users/10995026/Desktop/bhf-cvd-statistics---uk-factsheet%20\(2\).pdf](file:///C:/Users/10995026/Desktop/bhf-cvd-statistics---uk-factsheet%20(2).pdf)

BHF. (2017): [Online] [Accessed on July 10] <https://www.bhf.org.uk/research/heart-statistics/heart-statistics-publications/cardiovascular-disease-statistics-2017>

Bhoi, S., Verma, P., Vankar, S. and Galwankar, S. (2014) 'High sensitivity troponins and conventional troponins at the bedside.' *International Journal of Critical Illness and Injury Science*, 4(3), Jul-Sep, pp. 253-256.

Bingisser, R., Cairns, C., Christ, M., Hausfater, P., Lindahl, B., Mair, J., Panteghini, M., Price, C. and Venge, P. (2012) 'Cardiac troponin: a critical review of the case for point-of-care testing in the ED.' *American Journal of Emergency Medicine*, 30(8), Oct, pp. 1639-1649.

Body, R., McDowell, G., Carley, S. and Mackway-Jones, K. (2008) 'Do risk factors for chronic coronary heart disease help diagnose acute myocardial infarction in the Emergency Department?' *Resuscitation*, 79(1), Oct, pp. 41-45.

Body, R., Burrows, G., Carley, S. and Lewis, P. S. (2015a) 'The Manchester Acute Coronary Syndromes (MACS) decision rule: validation with a new automated assay for heart-type fatty acid binding protein.' *Emerg Med J*, 32(10), Oct, pp. 769-774.

Body, R., Burrows, G., Carley, S. and Lewis, P. S. (2015b) 'Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a sensitive troponin I assay.' *Ann Clin Biochem*, 52(Pt 5), Sep, pp. 543-549.

Body, R., Burrows, G., Carley, S. and Lewis, P. S. (2015c) 'Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a sensitive troponin I assay.' *Annals of Clinical Biochemistry*, 52(5), Sep, pp. 543-549.

Body, R., Cook, G., Burrows, G., Carley, S. and Lewis, P. S. (2014a) 'Can emergency physicians 'rule in' and 'rule out' acute myocardial infarction with clinical judgement?' *Emergency Medicine Journal*, 31(11), Nov, 2014/07/14, pp. 872-876.

Body, R., Carley, S., Wibberley, C., McDowell, G., Ferguson, J. and Mackway-Jones, K. (2010) 'The value of symptoms and signs in the emergent diagnosis of acute coronary syndromes.' *Resuscitation*, 81

Body, R., McDowell, G., Carley, S., Wibberley, C., Ferguson, J. and Mackway-Jones, K. (2011a) 'A FABP-ulous 'rule out' strategy? Heart fatty acid binding protein and troponin for rapid exclusion of acute myocardial infarction.' *Resuscitation*, 82(8), Aug, pp. 1041-1046.

Body, R., Carley, S., McDowell, G., Jaffe, A. S., France, M., Cruickshank, K., Wibberley, C., Nuttall, M. and Mackway-Jones, K. (2011b) 'Rapid Exclusion of Acute Myocardial Infarction in Patients With Undetectable Troponin Using a High-Sensitivity Assay.' *Journal of the American College of Cardiology*, 58(13), Sep 20, pp. 1333-1339.

Body, R., Carley, S., McDowell, G., Jaffe, A. S., France, M., Cruickshank, K., Wibberley, C., Nuttall, M. and Mackway-Jones, K. (2012) 'Rapid Exclusion of Acute Myocardial Infarction in Patients With Undetectable Troponin Using a High-Sensitivity Assay (vol 58, pg 1332, 2011).' *Journal of the American College of Cardiology*, 60(12), Sep 18, pp. 1122-1122.

Body, R., Carley, S., McDowell, G., Pemberton, P., Burrows, G., Cook, G., Lewis, P. S., Smith, A. and Mackway-Jones, K. (2014) 'The Manchester Acute Coronary Syndromes (MACS) decision rule for suspected cardiac chest pain: derivation and external validation.' *Heart*, April 29, 2014,

Body, R., Carley, S., McDowell, G., Pemberton, P., Burrows, G., Cook, G., Lewis, P. S., Smith, A. and Mackway-Jones, K. (2014b) 'The Manchester Acute Coronary Syndromes (MACS) decision rule for suspected cardiac chest pain: derivation and external validation.' *Heart*, 100(18), Sep 15, pp. 1462-1468.

Body, R., Carley, S., McDowell, G., Pemberton, P., Burrows, G., Cook, G., Lewis, P. S., Smith, A. and Mackway-Jones, K. (2014c) 'The Manchester

Acute Coronary Syndromes (MACS) decision rule for suspected cardiac chest pain: derivation and external validation.' *Heart*, 100(18), Sep, pp. 1462-1468.

Body, R., Carlton, E., Sperrin, M., Lewis, P. S., Burrows, G., Carley, S., McDowell, G., Buchan, I., Greaves, K. and Mackway-Jones, K. (2016a) 'Troponin-only Manchester Acute Coronary Syndromes (T-MACS) decision aid: single biomarker re-derivation and external validation in three cohorts.' *Emerg Med J*, Aug 26,

Body, R., Mueller, C., Giannitsis, E., Christ, M., Ordonez-Llanos, J., de Filippi, C. R., Nowak, R., Panteghini, M., Jernberg, T., Plebani, M., Verschuren, F., French, J. K., Christenson, R., Weiser, S., Bendig, G., Dilba, P. and Lindahl, B. (2016b) 'The Use of Very Low Concentrations of High-sensitivity Troponin T to Rule Out Acute Myocardial Infarction Using a Single Blood Test.' *Acad Emerg Med*, 23(9), Sep, 2016/05/15, pp. 1004-1013.

Brandt, D. R., Gates, R. C., Eng, K. K., Forsythe, C. M., Korom, G. K., Nitro, A. S., Koffler, P. A. and Ogunro, E. A. (1990) 'Quantifying the MB isoenzyme of creatine kinase with the Abbott "IMx" immunoassay analyzer.' *Clin Chem*, 36(2), Feb, pp. 375-378.

Bruno, P. (2011) 'The importance of diagnostic test parameters in the interpretation of clinical test findings: The Prone Hip Extension Test as an example.' *The Journal of the Canadian Chiropractic Association*, 55(2) pp. 69-75.

Buntinx, F. (2013) *New markers in suspected acute coronary syndromes: are they relevant for primary*

care? 6th Annual Scientific Meeting of the European Primary Care Cardiovascular Society London, United Kingdom: [Online] [Accessed on 8 August] <http://fabpulous.com/wp-content/uploads/2015/06/Buntinx-New-markers-Cardiovascular-disease-2013.pdf>

Burkett, E., Marwick, T., Thom, O. and Kelly, A.-M. (2014) 'A comparative analysis of risk stratification tools for emergency department patients with chest pain.' *International Journal of Emergency Medicine*, 7, 02/07

Carless, D. R., Wnek, M., Knox, C., Harrison, K. R., Calder, N., Hall, A. S. and Barth, J. H. (2013) 'Clinical and analytical evaluation of an immunoturbidimetric heart-type fatty acid-binding protein assay.' *Scandinavian Journal of Clinical & Laboratory Investigation*, 73(1), Feb, pp. 48-53.

Carlton, E., Greenslade, J., Cullen, L., Body, R., Than, M., Pickering, J. W., Aldous, S., Carley, S., Hammett, C., Kendall, J., Keevil, B., Lord, S.,

Parsonage, W. and Greaves, K. (2016) 'Evaluation of High-Sensitivity Cardiac Troponin I Levels in Patients With Suspected Acute Coronary Syndrome.' *JAMA Cardiol*, 1(4), Jul 01, pp. 405-412.

Carlton, E. W., Than, M., Cullen, L., Khattab, A. and Greaves, K. (2015a) 'Chest pain typicality' in suspected acute coronary syndromes and the impact of clinical experience.' *Am J Med*, 128(10), Oct, pp. 1109-1116 e1102.

Carlton, E. W., Cullen, L., Than, M., Gamble, J., Khattab, A. and Greaves, K. (2015b) 'A novel diagnostic protocol to identify patients suitable for discharge after a single high-sensitivity troponin.' *Heart*, 101(13), Jul 1, pp. 1041-1046.

Carroll, C., Al Khalaf, M., Stevens, J. W., Leaviss, J., Goodacre, S., Collinson, P. O. and Wang, J. (2013) 'Heart-type fatty acid binding protein as an early marker for myocardial infarction: systematic review and meta-analysis.' *Emergency Medicine Journal*, 30(4), Apr, pp. 280-286.

Cavus, U., Coskun, F., Yavuz, B., Ciftci, O., Sahiner, L., Aksoy, H., Deniz, A., Ozakin, E., Aytemir, K., Tokgozoglu, L. and Kabakci, G. (2006) 'Heart-type, fatty-acid binding protein can be a diagnostic marker in acute coronary syndromes.' *Journal of the National Medical Association*, 98(7), Jul, pp. 1067-1070.

Chan, D. W., Taylor, E., Frye, R. and Blitzler, R. L. (1985) 'Immunoenzymetric assay for creatine kinase MB with subunit-specific monoclonal antibodies compared with an immunochemical method and electrophoresis.' *Clin Chem*, 31(3), Mar, 1985/03/01, pp. 465-469.

Chandra, A., Lindsell, C. J., Limkakeng, A., Diercks, D. B., Hoekstra, J. W., Hollander, J. E., Kirk, J. D., Peacock, W. F., Gibler, W. B., Pollack, C. V. and Investigators, E. i. t. (2009) 'Emergency physician high pretest probability for acute coronary syndrome correlates with adverse cardiovascular outcomes.' *Acad Emerg Med*, 16(8), Aug, pp. 740-748.

Chapman, A., Adamson, P. and Mills, N. (2016) *Assessment and classification of patients with myocardial injury and infarction in clinical practice*. Vol. 103.

Chase, M., Robey, J. L., Zogby, K. E., Sease, K. L., Shofer, F. S. and Hollander, J. E. (2006) 'Prospective validation of the Thrombolysis in Myocardial Infarction Risk Score in the emergency department chest pain population.' *Ann Emerg Med*, 48(3), Sep, 2006/08/29, pp. 252-259.

Christenson, J., Innes, G., McKnight, D., Thompson, C. R., Wong, H., Yu, E., Boychuk, B., Grafstein, E., Rosenberg, F., Gin, K., Anis, A. and Singer, J.

(2006) 'A clinical prediction rule for early discharge of patients with chest pain.' *Annals of Emergency Medicine*, 47(1), Jan, pp. 1-10.

Christenson, R. H. and Azzazy, H. M. E. (2006) 'Biomarkers of Myocardial Necrosis.' In Morrow, D. A. (ed.) *Cardiovascular Biomarkers: Pathophysiology and Disease Management*. Totowa, NJ: Humana Press, pp. 3-25.

Collinson, P. (2015) 'Detecting cardiac events - state-of-the-art.' *Ann Clin Biochem*, 52(Pt 6), Nov, pp. 702-704.

Collinson, P., Hersey, J., Bray, R., Gaze, D., Lim, P., Firoozi, S., Ntalianis, A., Boa, F. and Prasad, A. (2017) 'Heart Fatty Acid Binding Protein for the Diagnosis of Myocardial Ischemia and Infarction.' *The Journal of Applied Laboratory Medicine: An AACC Publication*,

Collinson, P. O., Premachandram, S. and Hashemi, K. (2000) 'Prospective audit of incidence of prognostically important myocardial damage in patients discharged from emergency department.' *British Medical Journal*, 320(7251), Jun 24, pp. 1702-1704.

Cooper, A., Timmis, A., Skinner, J. and Grp, G. D. (2010) 'Guidelines Assessment of recent onset chest pain or discomfort of suspected cardiac origin: summary of NICE guidance.' *British Medical Journal*, 340, Mar 24,

Cullen, L., Mueller, C., Parsonage, W. A., Wildi, K., Greenslade, J. H., Twerenbold, R., Aldous, S., Meller, B., Tate, J. R., Reichlin, T., Hammett, C. J., Zellweger, C., Ungerer, J. P. J., Gimenez, M. R., Troughton, R., Murray, K., Brown, A. F. T., Mueller, M., George, P., Mosimann, T., Flaws, D. F., Reiter, M., Lamanna, A., Haaf, P., Pemberton, C. J., Richards, A. M., Chu, K., Reid, C. M., Peacock, W. F., Jaffe, A. S., Florkowski, C., Deely, J. M. and Than, M. (2013) 'Validation of High-Sensitivity Troponin I in a 2-Hour Diagnostic Strategy to Assess 30-Day Outcomes in Emergency Department Patients With Possible Acute Coronary Syndrome.' *Journal of the American College of Cardiology*, 62(14), Oct 1, pp. 1242-1249.

Cummins, B., Auckland, M. L. and Cummins, P. (1987) 'Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction.' *Am Heart J*, 113(6), Jun, 1987/06/01, pp. 1333-1344.

Damman, P., van't Hof, A. W., ten Berg, J. M., Jukema, J. W., Appelman, Y., Liem, A. H. and de Winter, R. J. (2017) '2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: comments from the Dutch ACS working group.' *Netherlands Heart Journal*, 25(3), Mar, pp. 181-185.

de Groot, M. J. M., Wodzig, K. W. H., Simoons, M. L., Glatz, J. F. C. and Hermens, W. T. (1999) 'Measurement of myocardial infarct size from plasma fatty acid-binding protein or myoglobin, using individually estimated clearance rates.' *Cardiovascular Research*, 44(2) pp. 315-324.

Dean, K. J. (1998) 'Biochemistry and Molecular Biology of Troponins I and T.' In Wu, A. H. B. (ed.) *Cardiac Markers*. Totowa, NJ: Humana Press, pp. 193-204.

Deeks, J. J. and Altman, D. G. (2004) 'Diagnostic tests 4: likelihood ratios.' *BMJ*, 329(7458) pp. 168-169.

Dey, S., Flather, M. D., Devlin, G., Brieger, D., Gurfinkel, E. P., Steg, P. G., FitzGerald, G., Jackson, E. A., Eagle, K. A. and Investigators, G. (2009) 'Sex-related differences in the presentation, treatment and outcomes among patients with acute coronary syndromes: the Global Registry of Acute Coronary Events.' *Heart*, 95(1), Jan, pp. 20-26.

Dezman, Z. D., Mattu, A. and Body, R. (2017) 'Utility of the History and Physical Examination in the Detection of Acute Coronary Syndromes in Emergency Department Patients.' *West J Emerg Med*, 18(4), Jun, pp. 752-760.

DG15, N. D. G. (2014) 'Myocardial infarction (acute): Early rule out using high-sensitivity troponin tests (Elecsys Troponin T high-sensitive, ARCHITECT STAT High Sensitive Troponin-I and AccuTnl+3 assays).' *National Institute for Health and Care Excellence*,

Diercks, D. B., Peacock, W. F., Hollander, J. E., Singer, A. J., Birkhahn, R., Shapiro, N., Glynn, T., Nowack, R., Safdar, B., Miller, C. D., Lewandrowski, E., Nagurney, J. T. and Investigators, M. (2012) 'Diagnostic accuracy of a point-of-care troponin I assay for acute myocardial infarction within 3 hours after presentation in early presenters to the emergency department with chest pain.' *American Heart Journal*, 163(1), Jan, pp. 74-U102.

Dupuy, A. M., Baillet, S., Dumont, R., Giraud, I., Badiou, S., Bargnoux, A. S., Kuster, N., Roubille, F. and Cristol, J. P. (2017) 'Point of Care Cardiac Troponin Assay Analytical Performances for their Use in Clinical Routine.' *Clinical Laboratory*, 63(4) pp. 851-854.

Ecollan, P., Collet, J. P., Boon, G., Tanguy, M. L., Fievet, M. L., Haas, R., Bertho, N., Siarni, S., Hubert, J. C., Coriat, P. and Montalescot, G. (2007) 'Pre-hospital detection of acute myocardial infarction with ultra-rapid human fatty acid-binding protein (H-FABP) immunoassay.' *Int J Cardiol*, 119(3), Jul 31, 2006/11/14, pp. 349-354.

Engel, J., Heeren, M. J., van der Wulp, I., de Bruijne, M. C. and Wagner, C. (2014) 'Understanding factors that influence the use of risk scoring instruments in the management of patients with unstable angina or non-ST-elevation myocardial infarction in the Netherlands: a qualitative study of health care practitioners' perceptions.' *Bmc Health Services Research*, 14, Sep 22,

Fabulous. (2016) 'Technology '

Faerstein, E. (2016) 'A DICTIONARY OF EPIDEMIOLOGY, 6th edition.' *Cadernos De Saude Publica*, 32(7), Jul,

Farah, C. S. and Reinach, F. C. (1995) 'The troponin complex and regulation of muscle contraction.' *Faseb j*, 9(9), Jun, 1995/06/01, pp. 755-767.

Figiel, L., Kasprzak, J. D., Peruga, J., Lipiec, P., Drozd, J., Krzeminska-Pakula, M. and Smigielski, J. (2008) 'Heart-type fatty acid binding protein - a reliable marker of myocardial necrosis in a heterogeneous group of patients with acute coronary syndrome without persistent ST elevation.' *Kardiologia Polska*, 66(3), Mar, pp. 253-259.

Fleiss, J. L., Levin, B. and Paik, M. C. (2013) *Statistical methods for rates and proportions*. John Wiley & Sons.

Fox, K. A., Birkhead, J., Wilcox, R., Knight, C. and Barth, J. (2004) 'British Cardiac Society Working Group on the definition of myocardial infarction.' *Heart*, 90(6), Jun, 2004/05/18, pp. 603-609.

Gami, B. N., Patel, D. S., Haridas, N., Chauhan, K. P., Shah, H. and Trivedi, A. (2015) 'Utility of Heart-type Fatty Acid Binding Protein as a New Biochemical Marker for the Early Diagnosis of Acute Coronary Syndrome.' *Journal of Clinical and Diagnostic Research : JCDR*, 9(1), 01/01

Gaze, D. C. (2016) 'Rapid Cardiovascular Diagnostics.' In Saxena, S. K. (ed.) *Proof and Concepts in Rapid Diagnostic Tests and Technologies*. Rijeka: InTech, p. Ch. 02.

Geeves, Michael A. and Lehrer, Sherwin S. (2014) 'Cross-Talk, Cross-Bridges, and Calcium Activation of Cardiac Contraction.' *Biophysical Journal*, 107(3), 05/28/received 06/09/accepted, pp. 543-545.

Glatz, J. F. and Renneberg, R. (2014) 'Added value of H-FABP as plasma biomarker for the early evaluation of suspected acute coronary syndrome.' *Clinical Lipidology*, 9(2) pp. 205-220.

Glatz, J. F., van der Vusse, G. J., Simoons, M. L., Kragten, J. A., van Dieijen-Visser, M. P. and Hermens, W. T. (1998) 'Fatty acid-binding protein and the early detection of acute myocardial infarction.' *Clin Chim Acta*, 272(1), Apr 06, pp. 87-92.

Glatz, J. F., Schaap, F. G., Binas, B., Bonen, A., van der Vusse, G. J. and Luiken, J. J. (2003) 'Cytoplasmic fatty acid-binding protein facilitates fatty acid utilization by skeletal muscle.' *Acta Physiol Scand*, 178(4), Aug, 2003/07/17, pp. 367-371.

Glatz, J. F. C. and Mohren, R. (2013) 'Plasma reference value of heart-type fatty acid-binding protein, the earliest available plasma biomarker of acute myocardial infarction.' *Health*, Vol.05No.08 p. 4.

Glatz, J. F. C., Vanbilsen, M., Paulussen, R. J. A., Veerkamp, J. H., Vandervusse, G. J. and Reneman, R. S. (1988) 'Release of Fatty Acid-Binding Protein from Isolated Rat-Heart Subjected to Ischemia and Reperfusion or to the Calcium Paradox.' *Biochimica Et Biophysica Acta*, 961(1), Jul 1, pp. 148-152.

Goodacre, S., Cross, E., Arnold, J., Angelini, K., Capewell, S. and Nicholl, J. (2005) 'The health care burden of acute chest pain.' *Heart*, 91(2), Feb, pp. 229-230.

Goodacre, S., Bradburn, M., Fitzgerald, P., Cross, E., Collinson, P., Gray, A. and Hall, A. S. (2011) 'The RATPAC (Randomised Assessment of Treatment using Panel Assay of Cardiac markers) trial: a randomised controlled trial of point-of-care cardiac markers in the emergency department.' *Health Technol Assess*, 15(23), May, 2011/05/28, pp. iii-xi, 1-102.

Goodacre, S., Thokala, P., Carroll, C., Stevens, J. W., Leaviss, J., Al Khalaf, M., Collinson, P., Morris, F., Evans, P. and Wang, J. (2013) 'Systematic review, meta-analysis and economic modelling of diagnostic strategies for suspected acute coronary syndrome.' *Health Technol Assess*, 17(1) pp. v-vi, 1-188.

Goodacre, S. W., Bradburn, M., Cross, E., Collinson, P., Gray, A., Hall, A. S., Arrowsmith, C., Barth, J., Bengner, J., Capewell, S., Chater, T., Coats, T., Cooper, C., Cooper, M., Coyle, J., Cross, L., Dixon, S., Fitzgerald, P., Gendall, E., Goodwin, E., Hall, K., Hassan, T., Humphrey, J., Julious, S., Kendall, J., Lawlor, V., Mackness, S., Meades, Y., Newby, D., Quartey, D., Robinson, K., Sibbick, G., Smith, J., Squire, R. and Team, R. R. (2011) 'The Randomised Assessment of Treatment using Panel Assay of Cardiac Markers (RATPAC) trial: a randomised controlled trial of point-of-care cardiac markers in the emergency department.' *Heart*, 97(3), Feb, pp. 190-196.

GRACE. (2001) 'Rationale and design of the GRACE (Global Registry of Acute Coronary Events) Project: a multinational registry of patients hospitalized with acute coronary syndromes.' *Am Heart J*, 141(2), Feb, 2001/02/15, pp. 190-199.

Granger, C. B., Goldberg, R. J., Dabbous, O., Pieper, K. S., Eagle, K. A., Cannon, C. P., Van De Werf, F., Avezum, A., Goodman, S. G., Flather, M. D. and Fox, K. A. (2003) 'Predictors of hospital mortality in the global registry of acute coronary events.' *Arch Intern Med*, 163(19), Oct 27, 2003/10/29, pp. 2345-2353.

Greenslade, J. H., Nayer, R., Parsonage, W., Doig, S., Young, J., Pickering, J. W., Than, M., Hammett, C. and Cullen, L. (2017) 'Validating the Manchester Acute Coronary Syndromes (MACS) and Troponin-only Manchester Acute Coronary Syndromes (T-MACS) rules for the prediction of acute myocardial infarction in patients presenting to the emergency department with chest pain.' *Emerg Med J*, Mar 31,

Gururajan, P., Gurumurthy, P., Nayar, P., Rao, G. S. N., Babu, S. and Cherian, K. M. (2010) 'Heart Fatty Acid Binding Protein (H-FABP) as a Diagnostic Biomarker in Patients with Acute Coronary Syndrome.' *Heart Lung and Circulation*, 19(11), Nov, pp. 660-664.

Halpern, E. J., Deutsch, J. P., Hannaway, M. M., Estepa, A. T., Kenia, A. S., Neuburger, K. J. and Levin, D. C. (2013) 'Cardiac risk factors and risk scores vs cardiac computed tomography angiography: a prospective cohort study for triage of ED patients with acute chest pain.' *American Journal of Emergency Medicine*, 31(10), Oct, pp. 1479-1485.

Haltern, G., Peiniger, S., Bufe, A., Reiss, G., Gulker, H. and Scheffold, T. (2010) 'Comparison of Usefulness of Heart-Type Fatty Acid Binding Protein Versus Cardiac Troponin T for Diagnosis of Acute Myocardial Infarction.' *American Journal of Cardiology*, 105(1), Jan 1, pp. 1-9.

Hamm, C. W., Bassand, J. P., Agewall, S., Bax, J., Boersma, E., Bueno, H., Caso, P., Dudek, D., Gielen, S., Huber, K., Ohman, M., Petrie, M. C., Sonntag, F., Uva, M. S., Storey, R. F., Wijns, W. and Zahger, D. (2011) 'ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC).' *Eur Heart J*, 32(23), Dec, 2011/08/30, pp. 2999-3054.

Hand, M. M. (1994) 'Emergency Department - Rapid Identification and Treatment of Patients with Acute Myocardial-Infarction.' *Annals of Emergency Medicine*, 23(2), Feb, pp. 311-329.

Hanna, E. B. and Glancy, D. L. (2015) 'ST-segment elevation: Differential diagnosis, caveats.' *Cleveland Clinic Journal of Medicine*, 82(6), Jun, pp. 373-384.

Hasić, S., Kiseljakovic, E., Jadrić, R., Radovanović, J. and Winterhalter-Jadrić, M. (2003) *Cardiac troponin I: the gold standard in acute myocardial infarction diagnosis*. Vol. 3.

Hawkins, R. C. (2007) 'Laboratory Turnaround Time.' *The Clinical Biochemist Reviews*, 28(4) pp. 179-194.

Henderson, A. R. and Bhayana, V. (1995) 'A Modest Proposal for the Consistent Presentation of Roc Plots in Clinical-Chemistry.' *Clinical Chemistry*, 41(8), Aug, pp. 1205-1206.

Hess, E. P. and Jaffe, A. S. (2012) 'Evaluation of Patients With Possible Cardiac Chest Pain A Way Out of the Jungle.' *Journal of the American College of Cardiology*, 59(23), Jun 5, pp. 2099-2100.

Hess, E. P., Brison, R. J., Perry, J. J., Calder, L. A., Thiruganasambandamoorthy, V., Agarwal, D., Sadosty, A. T., Silvilotti, M. L., Jaffe, A. S., Montori, V. M., Wells, G. A. and Stiell, I. G. (2012a) 'Development of a clinical prediction rule for 30-day cardiac events in emergency department patients with chest pain and possible acute coronary syndrome.' *Ann Emerg Med*, 59(2), Feb, 2011/09/03, pp. 115-125.e111.

Hess, E. P., Knoedler, M. A., Shah, N. D., Kline, J. A., Breslin, M., Branda, M. E., Pencille, L. J., Asplin, B. R., Nestler, D. M., Sadosty, A. T., Stiell, I. G., Ting, H. H. and Montori, V. M. (2012b) 'The chest pain choice decision aid: a randomized trial.' *Circ Cardiovasc Qual Outcomes*, 5(3), May, pp. 251-259.

Hess, E. P., Hollander, J. E., Schaffer, J. T., Kline, J. A., Torres, C. A., Diercks, D. B., Jones, R., Owen, K. P., Meisel, Z. F., Demers, M., Leblanc, A., Shah, N. D., Inselman, J., Herrin, J., Castaneda-Guarderas, A. and Montori, V. M. (2016) 'Shared decision making in patients with low risk chest pain: prospective randomized pragmatic trial.' *The BMJ*, 355, 12/05 11/03/accepted, p. i6165.

Hillis, G. S., Zhao, N., Taggart, P., Dalsey, W. C. and Mangione, A. (1999) 'Utility of cardiac troponin I, creatine kinase-MBmass, myosin light chain 1, and

myoglobin in the early in-hospital triage of "high risk" patients with chest pain.' *Heart*, 82(5), Nov, pp. 614-620.

Hlatky and Hong, Y. L. (2009) 'Criteria for Evaluation of Novel Markers of Cardiovascular Risk: A Scientific Statement From the American Heart Association (vol 119, pg 2408, 2009).' *Circulation*, 119(25), Jun 30, pp. E606-E606.

Hobbs, R. and Arroll, B. (2009) 'Cardiovascular Risk Management.'

Hollander, J. E. (1999) 'The continuing search to identify the very-low-risk chest pain patient.' *Academic Emergency Medicine*, 6(10), Oct, pp. 979-981.

Huma, S., Tariq, R., Amin Dr, F. and Mahmood Dr, K. T. (2012) 'Modifiable and non-modifiable predisposing risk factors of myocardial infarction -A review.' *J. Pharm. Sci. Res. Journal of Pharmaceutical Sciences and Research*, 4(1) pp. 1649-1653.

Ibanez, B., James, S., Agewall, S., Antunes, M. J., Bucciarelli-Ducci, C., Bueno, H., Caforio, A. L. P., Crea, F., Goudevenos, J. A., Halvorsen, S., Hindricks, G., Kastrati, A., Lenzen, M. J., Prescott, E., Roffi, M., Valgimigli, M., Varenhorst, C., Vranckx, P., Widimský, P., Collet, J.-P., Kristensen, S. D., Aboyans, V., Baumbach, A., Bugiardini, R., Coman, I. M., Delgado, V., Fitzsimons, D., Gaemperli, O., Gershlick, A. H., Gielen, S., Harjola, V.-P., Katus, H. A., Knuuti, J., Kolh, P., Leclercq, C., Lip, G. Y. H., Morais, J., Neskovic, A. N., Neumann, F.-J., Niessner, A., Piepoli, M. F., Richter, D. J., Shlyakhto, E., Simpson, I. A., Steg, P. G., Terkelsen, C. J., Thygesen, K., Windecker, S., Zamorano, J. L., Zeymer, U., Windecker, S., Aboyans, V., Agewall, S., Barbato, E., Bueno, H., Coca, A., Collet, J.-P., Coman, I. M., Dean, V., Delgado, V., Fitzsimons, D., Gaemperli, O., Hindricks, G., Iung, B., Jüni, P., Katus, H. A., Knuuti, J., Lancellotti, P., Leclercq, C., McDonagh, T., Piepoli, M. F., Ponikowski, P., Richter, D. J., Roffi, M., Shlyakhto, E., Simpson, I. A., Zamorano, J. L., Chettibi, M., Hayrapetyan, H. G., Metzler, B., Ibrahimov, F., Sujayeva, V., Beauloye, C., Dizdarevic-Hudic, L., Karamfiloff, K., Skoric, B., Antoniades, L., Tousek, P., Terkelsen, P. J., Shaheen, S. M., Marandi, T., Niemelä, M., Kedev, S., Gilard, M., Aladashvili, A., Elsaesser, A., Kanakakis, I. G., Merkely, B., Gudnason, T., Iakobishvili, Z., Bolognese, L., Berkinbayev, S., Bajraktari, G., Beishenkulov, M., Zake, I., Lamin, H. B., Gustiene, O., Pereira, B., Xuereb, R. G., Ztot, S., Juliebø, V., Legutko, J., Timóteo, A. T., Tatu-Chițoiu, G., Yakovlev, A., Bertelli, L., Nedeljkovic, M., Studenčan, M., Bunc, M., García de Castro, A. M., Petursson, P., Jeger, R., Murali, M. S., Yildirim, A., Parkhomenko, A. and Gale, C. P. (2017) '2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevationThe Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC).' *European Heart Journal*, pp. ehx393-ehx393.

Jaffe, A. S. (2013) 'Third Universal Definition of Myocardial Infarction.' *Clinical Biochemistry*, 46(1-2), Jan, pp. 1-4.

Jaffe, A. S. and Apple, F. S. (2012a) 'The Third Universal Definition of Myocardial Infarction-Moving Forward.' *Clinical Chemistry*, 58(12), Dec, pp. 1727-1728.

Jaffe, A. S. and Apple, F. S. (2012b) 'The third Universal Definition of Myocardial Infarction--moving forward.' *Clin Chem*, 58(12), Dec, pp. 1727-1728.

Jaffery, Z., Hudson, M. P., Jacobsen, G., Nowak, R. and McCord, J. (2007) 'Modified thrombolysis in myocardial infarction (TIMI) risk score to risk stratify patients in the emergency department with possible acute coronary syndrome.' *J Thromb Thrombolysis*, 24(2), Oct, pp. 137-144.

James, S. K., Lindahl, B., Armstrong, P., Califf, R., Simoons, M. L., Venge, P. and Wallentin, L. (2004) 'A rapid troponin I assay is not optimal for determination of troponin status and prediction of subsequent cardiac events at suspicion of unstable coronary syndromes.' *Int J Cardiol*, 93(2-3), Feb, 2004/02/21, pp. 113-120.

Jangaard, N., Sarkisian, L., Saaby, L., Mikkelsen, S., Lassen, A. M., Marcussen, N., Thomsen, J. L., Diederichsen, A. C. P., Thygesen, K. and Mickley, H. (2017) 'Incidence, Frequency, and Clinical Characteristics of Type 3 Myocardial Infarction in Clinical Practice.' *Am J Med*, 130(7), Jul, pp. 862 e869-862 e814.

Jokhadar, M. and Wenger, N. K. (2009) 'Review of the treatment of acute coronary syndrome in elderly patients.' *Clinical Interventions in Aging*, 4, 11/18 11/16/received, pp. 435-444.

Jones, J. D., Chew, P. G., Dobson, R., Wootton, A., Ashrafi, R. and Khand, A. (2017) 'The Prognostic Value of Heart Type Fatty Acid Binding Protein in Patients with Suspected Acute Coronary Syndrome: A Systematic Review.' *Curr Cardiol Rev*, 13(3) 2017/01/18, pp. 189-198.

Katus, H. A., Remppis, A., Looser, S., Hallermeier, K., Scheffold, T. and Kubler, W. (1989) 'Enzyme linked immuno assay of cardiac troponin T for the detection of acute myocardial infarction in patients.' *J Mol Cell Cardiol*, 21(12), Dec, pp. 1349-1353.

Kavsak, P. and Jaffe, A. S. (2009) 'Sensitive cardiac troponin assays.' *N Engl J Med*, 361(26), Dec 24, 2010/01/06, pp. 2575-2576; author reply 2576-2577.

Keffer, J. H. (1997) *Recent advances in myocardial markers of injury*. Philadelphia, Pa.: W.B. Saunders.

Kellens, S., Verbrugge, F. H., Vanmechelen, M., Grieten, L., Van Lierde, J., Dens, J., Vrolix, M. and Vandervoort, P. (2016) 'Point-of-care heart-type fatty acid binding protein versus high-sensitivity troponin T testing in emergency patients at high risk for acute coronary syndrome.' *European Heart Journal-Acute Cardiovascular Care*, 5(2), Apr, pp. 177-184.

Keller, T., Zeller, T., Ojeda, F., Tzikas, S., Lillpopp, L., Sinning, C., Wild, P., Genth-Zotz, S., Warnholtz, A., Giannitsis, E., Mockel, M., Bickel, C., Peetz, D., Lackner, K., Baldus, S., Munzel, T. and Blankenberg, S. (2011a) 'Serial changes in highly sensitive troponin I assay and early diagnosis of myocardial infarction.' *JAMA*, 306(24), Dec 28, pp. 2684-2693.

Keller, T., Zeller, T., Ojeda, F., Tzikas, S., Lillpopp, L., Sinning, C., Wild, P., Genth-Zotz, S., Warnholtz, A., Giannitsis, E., Mockel, M., Bickel, C., Peetz, D., Lackner, K., Baldus, S., Munzel, T. and Blankenberg, S. (2011b) 'Serial Changes in Highly Sensitive Troponin I Assay and Early Diagnosis of Myocardial Infarction.' *Jama-Journal of the American Medical Association*, 306(24), Dec 28, pp. 2684-2693.

Kha, K. S. and Chien, P. F. (2001) 'Evaluation of a clinical test. I: assessment of reliability.' *BJOG: An International Journal of Obstetrics & Gynaecology*, 108(6) pp. 562-567.

Khalill, R., Han, L., Jing, C. and Quan, H. (2009) 'The use of risk scores for stratification of non-ST elevation acute coronary syndrome patients.' *Experimental & Clinical Cardiology*, 14(2), Summer

Kienle, G. S. and Kiene, H. (2011) 'Clinical judgement and the medical profession.' *Journal of Evaluation in Clinical Practice*, 17(4), Aug, pp. 621-627.

Kline, J. A. and Stubblefield, W. B. (2014) 'Clinician gestalt estimate of pretest probability for acute coronary syndrome and pulmonary embolism in patients with chest pain and dyspnea.' *Ann Emerg Med*, 63(3), Mar, pp. 275-280.

Kontos, M. C., Diercks, D. B. and Kirk, J. D. (2010) 'Emergency Department and Office-Based Evaluation of Patients With Chest Pain.' *Mayo Clinic Proceedings*, 85(3) pp. 284-299.

Kratz, A., Januzzi, J. L., Lewandrowski, K. B. and Lee-Lewandrowski, E. (2002) 'Positive predictive value of a point-of-care testing strategy on first-draw specimens for the emergency department-based detection of acute coronary

syndromes.' *Archives of Pathology & Laboratory Medicine*, 126(12), Dec, pp. 1487-1493.

Krone, R. (2007) 'Cardiovascular Biomarkers edited by David A. Morrow.' *Journal of interventional cardiology*, 20(1) pp. 89-90.

Kumar, A. and Cannon, C. P. (2009) 'Acute Coronary Syndromes: Diagnosis and Management, Part I.' *Mayo Clinic Proceedings*, 84(10) pp. 917-938.

Ladue, J. S., Wroblewski, F. and Karmen, A. (1954) 'Serum glutamic oxaloacetic transaminase activity in human acute transmural myocardial infarction.' *Science*, 120(3117), Sep 24, 1954/09/24, pp. 497-499.

Landis, J. R. and Koch, G. G. (1977) 'The measurement of observer agreement for categorical data.' *Biometrics*, 33(1), Mar, 1977/03/01, pp. 159-174.

Larsson, A., Greig-Pylypczuk, R. and Huisman, A. (2015) 'The state of point-of-care testing: a European perspective.' *Ups J Med Sci*, 120(1), Mar, pp. 1-10.

Lee-Lewandrowski, E., Januzzi, J. L., Grisson, R., Mohammed, A. A., Lewandrowski, G. and Lewandrowski, K. (2011a) 'Evaluation of First-Draw Whole Blood, Point-of-Care Cardiac Markers in the Context of the Universal Definition of Myocardial Infarction A Comparison of a Multimarker Panel to Troponin Alone and to Testing in the Central Laboratory.' *Archives of Pathology & Laboratory Medicine*, 135(4), Apr, pp. 459-463.

Lee-Lewandrowski, E., Januzzi, J. L., Jr., Grisson, R., Mohammed, A. A., Lewandrowski, G. and Lewandrowski, K. (2011b) 'Evaluation of first-draw whole blood, point-of-care cardiac markers in the context of the universal definition of myocardial infarction: a comparison of a multimarker panel to troponin alone and to testing in the central laboratory.' *Arch Pathol Lab Med*, 135(4), Apr, pp. 459-463.

Leite, L., Baptista, R., Leitao, J., Cochicho, J., Breda, F., Elvas, L., Fonseca, I., Carvalho, A. and Costa, J. N. (2015) 'Chest pain in the emergency department: risk stratification with Manchester triage system and HEART score.' *Bmc Cardiovascular Disorders*, 15, Jun 11,

Liebetrau, C., Nef, H. M., Dorr, O., Gaede, L., Hoffmann, J., Hahnel, A., Rolf, A., Troidl, C., Lackner, K. J., Keller, T., Hamm, C. W. and Mollmann, H. (2014) 'Release kinetics of early ischaemic biomarkers in a clinical model of acute myocardial infarction.' *Heart*, 100(8), Apr, 2014/02/04, pp. 652-657.

Lilly, L. S. and Braunwald, E. (2015) *Braunwald's heart disease : review and assessment*.

Lilly, L. S. and Harvard Medical, S. (2016) *Pathophysiology of heart disease : a collaborative project of medical students and faculty*.

Lippi, G., Mattiuzzi, C. and Cervellin, G. (2013) 'Critical review and meta-analysis on the combination of heart-type fatty acid-binding protein (H-FABP) and troponin for early diagnosis of acute myocardial infarction.' *Clin Biochem*, 46

Lippi, G., Montagnana, M., Salvagno, G. L. and Guidi, G. C. (2006) 'Potential value for new diagnostic markers in the early recognition of acute coronary syndromes.' *CJEM*, 8(1), Jan, pp. 27-31.

Long, B. (2016) *The Great and Powerful HEART Score: Does it have a weakness?* : [Online] [Accessed on 17 August] <http://www.emdocs.net/great-powerful-heart-score-weakness/>

Looney, S. W. (2002) *Biostatistical methods*. Totowa, NJ: Humana Press.

Lopez-Cuenca, A., Gomez-Molina, M., Flores-Blanco, P. J., Sanchez-Martinez, M., Garcia-Narbon, A., Las Heras-Gomez, I., Sanchez-Galian, M. J., Guerrero-Perez, E., Valdes, M. and Manzano-Fernandez, S. (2016) 'Comparison between type-2 and type-1 myocardial infarction: clinical features, treatment strategies and outcomes.' *Journal of Geriatric Cardiology*, 13(1) pp. 15-22.

Lopez, B., Sanchez, M., Bragulat, E., Jimenez, S., Coll-Vinent, B., Ortega, M., Gomez-Angelats, E. and Miro, O. (2011) 'Validation of a triage flowchart to rule out acute coronary syndrome.' *Emerg Med J*, 28(10), Oct, pp. 841-846.

Lum, G., Solarz, D. E. and Farney, L. (2006) 'False positive cardiac troponin results in patients without acute myocardial infarction.' *Labmedicine*, 37(9), Sep, pp. 546-550.

Luppa, P. B., Müller, C., Schlichtiger, A. and Schlebusch, H. (2011) 'Point-of-care testing (POCT): Current techniques and future perspectives.' *TrAC Trends in Analytical Chemistry*, 30(6), 2011/06/01/, pp. 887-898.

Lux, R. L. (2017) 'Basis and ECG measurement of global ventricular repolarization.' *J Electrocardiol*, Jul 12,

Mad, P., Domanovits, H., Fazelnia, C., Stiassny, K., Russmuller, G., Cseh, A., Sodeck, G., Binder, T., Christ, G., Szekeres, T., Laggner, A. and Herkner, H. (2007a) 'Human heart-type fatty-acid-binding protein as a point-of-care test in the early diagnosis of acute myocardial infarction.' *Qjm-an International Journal of Medicine*, 100(4), Apr, pp. 203-210.

Mad, P., Domanovits, H., Fazelnia, C., Stiassny, K., Russmüller, G., Cseh, A., Sodeck, G., Binder, T., Christ, G., Szekeres, T., Laggner, A. and Herkner, H. (2007b) 'Human heart-type fatty-acid-binding protein as a point-of-care test in the early diagnosis of acute myocardial infarction.' *QJM: An International Journal of Medicine*, 100(4) pp. 203-210.

Mahler, S. A., Riley, R. F., Hiestand, B. C., Russell, G. B., Hoekstra, J. W., Lefebvre, C. W., Nicks, B. A., Cline, D. M., Askew, K. L., Elliott, S. B., Herrington, D. M., Burke, G. L. and Miller, C. D. (2015) 'The HEART Pathway randomized trial: identifying emergency department patients with acute chest pain for early discharge.' *Circ Cardiovasc Qual Outcomes*, 8(2), Mar, 2015/03/05, pp. 195-203.

Mahler, S. A., Stopyra, J. P., Apple, F. S., Riley, R. F., Russell, G. B., Hiestand, B. C., Hoekstra, J. W., Lefebvre, C. W., Nicks, B. A., Cline, D. M., Askew, K. L., Herrington, D. M., Burke, G. L. and Miller, C. D. (2017) 'Use of the HEART Pathway with high sensitivity cardiac troponins: A secondary analysis.' *Clin Biochem*, 50(7-8), May, 2017/01/15, pp. 401-407.

Mair, J., Artner-Dworzak, E., Lechleitner, P., Morass, B., Smidt, J., Wagner, I., Dienstl, F. and Puschendorf, B. (1992) 'Early diagnosis of acute myocardial infarction by a newly developed rapid immunoturbidimetric assay for myoglobin.' *Br Heart J*, 68(5), Nov, pp. 462-468.

Maisel, A. S. and Jaffe, A. S. (2016) 'Cardiac biomarkers : case studies and clinical correlations.'

McCann, C. J., Glover, B. M., Menown, I. B. A., Moore, M. J., McEneny, J., Owens, C. G., Smith, B., Sharpe, P. C., Young, I. S. and Adgey, J. A. (2008) 'Novel biomarkers in early diagnosis of acute myocardial infarction compared with cardiac troponin T.' *European Heart Journal*, 29(23), Dec, pp. 2843-2850.

McCord, J., Nowak, R. M., McCullough, P. A., Foreback, C., Borzak, S., Tokarski, G., Tomlanovich, M. C., Jacobsen, G. and Weaver, W. D. (2001) 'Ninety-minute exclusion of acute myocardial infarction by use of quantitative point-of-care testing of myoglobin and troponin I.' *Circulation*, 104(13), Sep 25, pp. 1483-1488.

McMahon, C. G., Lamont, J. V., Curtin, E., McConnell, R. I., Crockard, M., Kurth, M. J., Crean, P. and Fitzgerald, S. P. (2012a) 'Diagnostic accuracy of

heart-type fatty acid-binding protein for the early diagnosis of acute myocardial infarction.' *Am J Emerg Med*, 30(2), Feb, 2011/01/07, pp. 267-274.

McMahon, C. G., Lamont, J. V., Curtin, E., McConnell, R. I., Crockard, M., Kurth, M. J., Crean, P. and Fitzgerald, S. P. (2012b) 'Diagnostic accuracy of heart-type fatty acid-binding protein for the early diagnosis of acute myocardial infarction.' *Am J of Emergency Med*, 30

Melanson, S. E. F., Tanasijevic, M. J. and Jarolim, P. (2007) 'Cardiac Troponin Assays.' *A View From the Clinical Chemistry Laboratory*, 116(18) pp. e501-e504.

Milosevic, A., Vasiljevic-Pokrajcic, Z., Milasinovic, D., Marinkovic, J., Vukcevic, V., Stefanovic, B., Asanin, M., Dikic, M., Stankovic, S. and Stankovic, G. (2016) 'Immediate Versus Delayed Invasive Intervention for Non-STEMI Patients The RIDDLE-NSTEMI Study.' *Jacc-Cardiovascular Interventions*, 9(6), Mar 28, pp. 541-549.

Mion, M. M., Novello, E., Altinier, S., Rocco, S., Zaninotto, M. and Plebani, M. (2007) 'Analytical and clinical performance of a fully automated cardiac multi-markers strategy based on protein biochip microarray technology.' *Clinical Biochemistry*, 40(16-17), Nov, pp. 1245-1251.

Mokhtari, A., Dryver, E., Soderholm, M. and Ekelund, U. (2015) 'Diagnostic values of chest pain history, ECG, troponin and clinical gestalt in patients with chest pain and potential acute coronary syndrome assessed in the emergency department.' *Springerplus*, 4 p. 219.

Mueller, C. (2014) 'Biomarkers and acute coronary syndromes: an update.' *European Heart Journal*, 35(9), Mar, pp. 552-U513.

Mueller, M., Biener, M., Vafaie, M., Doerr, S., Keller, T., Blankenberg, S., Katus, H. A. and Giannitsis, E. (2012) 'Absolute and relative kinetic changes of high-sensitivity cardiac troponin T in acute coronary syndrome and in patients with increased troponin in the absence of acute coronary syndrome.' *Clin Chem*, 58(1), Jan, 2011/12/03, pp. 209-218.

Mythili, S. and Malathi, N. (2015) 'Diagnostic markers of acute myocardial infarction.' *Biomedical Reports*, 3(6), Nov, pp. 743-748.

Nagurney, J. T., Brown, D. F., Chae, C., Chang, Y., Chung, W. G., Cranmer, H., Dan, L., Fisher, J., Grossman, S., Tedrow, U., Lewandowski, K. and Jang, I. K. (2005) 'The sensitivity of cardiac markers stratified by symptom duration.' *J Emerg Med*, 29(4), Nov, 2005/10/26, pp. 409-415.

NICE. (2010) NICE Clinical Guideline 95. Chest pain of recent onset. National Clinical Guideline Centre for Acute and Chronic Conditions. 2010 [www.nice.org.uk/nicemedia/live/12947/47938/47938pdf].

NIH. (April 25, 2013) *What Is Atherosclerosis?* : National Institutes of Health. [Online] [Accessed on 15 August] <https://www.nhlbi.nih.gov/health/health-topics/topics/atherosclerosis>

Niizeki, T., Takeishi, Y., Takabatake, N., Shibata, Y., Konta, T., Kato, T., Kawata, S. and Kubota, I. (2007) 'Circulating levels of heart-type fatty acid-binding protein in a general Japanese population: effects of age, gender, and physiologic characteristics.' *Circ J*, 71(9), Sep, 2007/08/28, pp. 1452-1457.

O'Donoghue, M., de Lemos, J. A., Morrow, D. A., Murphy, S. A., Buross, J. L., Cannon, C. P. and Sabatine, M. S. (2006) 'Prognostic utility of heart-type fatty acid binding protein in patients with acute coronary syndromes.' *Circulation*, 114(6), Aug 8, pp. 550-557.

Palamalai, V., Murakami, M. M. and Apple, F. S. (2013) 'Diagnostic performance of four point of care cardiac troponin I assays to rule in and rule out acute myocardial infarction.' *Clinical Biochemistry*, 46(16-17), Nov, pp. 1631-1635.

Parikh, R., Mathai, A., Parikh, S., Sekhar, G. C. and Thomas, R. (2008) 'Understanding and using sensitivity, specificity and predictive values - Reply.' *Indian Journal of Ophthalmology*, 56(4), Jul-Aug, pp. 341-341.

Peacock, W. F., Diercks, D., Birkhahn, R., Singer, A. J., Hollander, J. E., Nowak, R., Safdar, B., Miller, C. D., Peberdy, M., Counsellman, F., Chandra, A., Kosowsky, J., Neuenschwander, J., Schrock, J., Lee-Lewandrowski, E., Arnold, W. and Nagurny, J. (2016) 'Can a Point-of-Care Troponin I Assay be as Good as a Central Laboratory Assay? A MIDAS Investigation.' *Annals of Laboratory Medicine*, 36(5), 06/24

Penttila, I., Penttila, K. and Rantanen, T. (2000) 'Laboratory diagnosis of patients with acute chest pain.' *Clin Chem Lab Med*, 38(3), Mar, pp. 187-197.

Peters, R. J. G., Mehta, S. and Yusuf, S. (2007) 'Acute coronary syndromes without ST segment elevation.' *BMJ*, 334(7606) pp. 1265-1269.

Pickering, J. W., Than, M. P., Cullen, L., Aldous, S., Ter Avest, E., Body, R., Carlton, E. W., Collinson, P., Dupuy, A. M., Ekelund, U., Eggers, K. M., Florkowski, C. M., Freund, Y., George, P., Goodacre, S., Greenslade, J. H., Jaffe, A. S., Lord, S. J., Mokhtari, A., Mueller, C., Munro, A., Mustapha, S.,

Parsonage, W., Peacock, W. F., Pemberton, C., Richards, A. M., Sanchis, J., Staub, L. P., Troughton, R., Twerenbold, R., Wildi, K. and Young, J. (2017) 'Rapid Rule-out of Acute Myocardial Infarction With a Single High-Sensitivity Cardiac Troponin T Measurement Below the Limit of Detection: A Collaborative Meta-analysis.' *Ann Intern Med*, 166(10), May 16, 2017/04/19, pp. 715-724.

Pines, J. M., Pollack, C. V., Jr., Diercks, D. B., Chang, A. M., Shofer, F. S. and Hollander, J. E. (2009) 'The association between emergency department crowding and adverse cardiovascular outcomes in patients with chest pain.' *Acad Emerg Med*, 16(7), Jul, 2009/06/25, pp. 617-625.

Pinnell, J., Turner, S. and Howell, S. (2007) 'Cardiac muscle physiology.' *Continuing Education in Anaesthesia Critical Care & Pain*, 7(3) pp. 85-88.

Plüddemann, A., Heneghan, C., Thompson, M., Roberts, N., Summerton, N., Linden-Phillips, L., Packer, C. and Price, C. P. (2010) 'Prioritisation criteria for the selection of new diagnostic technologies for evaluation.' *BMC Health Services Research*, 10(1), May 05, p. 109.

Poldervaart, J. M., Reitsma, J. B., Backus, B. E. and et al. (2017) 'Effect of using the heart score in patients with chest pain in the emergency department: A stepped-wedge, cluster randomized trial.' *Annals of Internal Medicine*, 166(10) pp. 689-697.

Pollack, C. V., Sites, F. D., Shofer, F. S., Sease, K. L. and Hollander, J. E. (2006) 'Application of the TIMI risk score for unstable angina and non-ST elevation acute coronary syndrome to an unselected emergency department chest pain population.' *Academic Emergency Medicine*, 13(1), Jan, pp. 13-18.

Pope, J. H., Aufderheide, T. P., Ruthazer, R., Woolard, R. H., Feldman, J. A., Beshansky, J. R., Griffith, J. L. and Selker, H. P. (2000) 'Missed diagnoses of acute cardiac ischemia in the emergency department.' *N Engl J Med*, 342(16), Apr 20, pp. 1163-1170.

Puleo, P. R., Guadagno, P. A., Roberts, R., Scheel, M. V., Marian, A. J., Churchill, D. and Perryman, M. B. (1990) 'Early diagnosis of acute myocardial infarction based on assay for subforms of creatine kinase-MB.' *Circulation*, 82(3), Sep, 1990/09/01, pp. 759-764.

Puleo, P. R., Meyer, D., Wathen, C., Tawa, C. B., Wheeler, S., Hamburg, R. J., Ali, N., Obermueller, S. D., Triana, F. J., Zimmerman, J. L., Perryman, M. B. and Roberts, R. (1994) 'Use of a Rapid Assay of Subforms of Creatine Kinase MB to Diagnose or Rule Out Acute Myocardial Infarction.' *New England Journal of Medicine*, 331(9) pp. 561-566.

Pyati, A. K., Devaranavadagi, B. B., Sajjannar, S. L., Nikam, S. V., Shannawaz, M. and Patil, S. (2016) 'Heart-Type Fatty Acid-Binding Protein, in Early Detection of Acute Myocardial Infarction: Comparison with CK-MB, Troponin I and Myoglobin.' *Indian J Clin Biochem*, 31(4), Oct, pp. 439-445.

Quinn, A. D., Dixon, D. and Meenan, B. J. (2016) 'Barriers to hospital-based clinical adoption of point-of-care testing (POCT): A systematic narrative review.' *Crit Rev Clin Lab Sci*, 53(1) pp. 1-12.

Radovanovic, D., Pilgrim, T., Seifert, B., Urban, P., Pedrazzini, G. and Erne, P. (2017) 'Type 2 myocardial infarction: incidence, presentation, treatment and outcome in routine clinical practice.' *Journal of Cardiovascular Medicine*, 18(5), May, pp. 341-347.

RANDOX. (2015) *What is H-FABP?* : [Online] [Accessed <http://www.h-fabp.com/what-is-h-fabp/>]

Rathore, S., Knowles, P., Mann, A. P. and Dodds, P. A. (2008) 'Is it safe to discharge patients from accident and emergency using a rapid point of care Triple Cardiac Marker test to rule out acute coronary syndrome in low to intermediate risk patients presenting with chest pain?' *Eur J Intern Med*, 19(7), Nov, 2008/11/18, pp. 537-540.

Reddy, L. L., Shah, S. A. V., Dherai, A. J., Ponde, C. K. and Ashavaid, T. F. (2016) 'Troponin T and Heart Type Fatty Acid Binding Protein (h-Fabp) as Biomarkers in Patients Presenting with Chest Pain.' *Indian Journal of Clinical Biochemistry*, 31(1), 03/14

Reichlin, T., Hochholzer, W., Bassetti, S., Steuer, S., Stelzig, C., Hartwiger, S., Biedert, S., Schaub, N., Buerge, C., Potocki, M., Noveanu, M., Breidthardt, T., Twerenbold, R., Winkler, K., Bingisser, R. and Mueller, C. (2009) 'Early diagnosis of myocardial infarction with sensitive cardiac troponin assays.' *N Engl J Med*, 361(9), Aug 27, pp. 858-867.

Reichlin, T., Twerenbold, R., Wildi, K., Gimenez, M. R., Bergsma, N., Haaf, P., Druey, S., Puelacher, C., Moehring, B., Freese, M., Stelzig, C., Krivoshei, L., Hillinger, P., Jäger, C., Herrmann, T., Kreutzinger, P., Radosavac, M., Weidmann, Z. M., Pershyna, K., Honegger, U., Wagener, M., Vuillomenet, T., Campodarve, I., Bingisser, R., Miró, Ò., Rentsch, K., Bassetti, S., Osswald, S. and Mueller, C. (2015) 'Prospective validation of a 1-hour algorithm to rule-out and rule-in acute myocardial infarction using a high-sensitivity cardiac troponin T assay.' *Canadian Medical Association Journal*, April 13, 2015,

Reiter, M., Twerenbold, R., Reichlin, T., Haaf, P., Peter, F., Meissner, J., Hochholzer, W., Stelzig, C., Freese, M., Heinisch, C., Breidthardt, T., Freidank,

H., Winkler, K., Campodarve, I., Gea, J. and Mueller, C. (2011) 'Early diagnosis of acute myocardial infarction in the elderly using more sensitive cardiac troponin assays.' *Eur Heart J*, 32(11), Jun, 2011/03/03, pp. 1379-1389.

Rhee, J., Jo, Y., Kim, T., Lee, J., Kim, K., Suh, G., Lee, H., Lee, W. and Kim, M. (2008) 'The Quantitative Point-of-Care Test of Heart-Type Fatty Acid-Binding Protein for the Early Diagnosis of Acute Myocardial Infarction.' *Annals of Emergency Medicine*, 52(4), Oct, pp. S117-S117.

Riley, R. F., Miller, C. D., Russell, G. B., Harper, E. N., Hiestand, B. C., Hoekstra, J. W., Lefebvre, C. W., Nicks, B. A., Cline, D. M., Askew, K. L. and Mahler, S. A. (2017) 'Cost analysis of the History, ECG, Age, Risk factors, and initial Troponin (HEART) Pathway randomized control trial.' *Am J Emerg Med*, 35(1), Jan, 2016/10/22, pp. 77-81.

Rising, K. L., Hollander, J. E., Schaffer, J. T., Kline, J. A., Torres, C. A., Diercks, D. B., Jones, R., Owen, K. P., Meisel, Z. F., Demers, M., Leblanc, A., Shah, N. D., Inselman, J., Herrin, J., Montori, V. M. and Hess, E. P. (2017) 'Effectiveness of a Decision Aid in Potentially Vulnerable Patients: A Secondary Analysis of the Chest Pain Choice Multicenter Randomized Trial.' *Med Decis Making*, May 01, p. 272989X17706363.

Roffi, M., Patrono, C., Collet, J. P., Mueller, C., Valgimigli, M., Andreotti, F., Bax, J. J., Borger, M. A., Brotons, C., Chew, D. P., Gencer, B., Hasenfuss, G., Kjeldsen, K., Lancellotti, P., Landmesser, U., Mehilli, J., Mukherjee, D., Storey, R. F. and Windecker, S. (2015) '2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation Working Group of the European Society of Cardiology (ESC) to conduct affairs in acute coronary syndromes without ST segment elevation of persistent.' *Kardiologia Polska*, 73(12) pp. 1207-1294.

Roffi, M., Patrono, C., Collet, J. P., Mueller, C., Valgimigli, M., Andreotti, F., Bax, J. J., Borger, M. A., Brotons, C., Chew, D. P., Gencer, B., Hasenfuss, G., Kjeldsen, K., Lancellotti, P., Landmesser, U., Mehilli, J., Mukherjee, D., Storey, R. F., Windecker, S., Baumgartner, H., Gaemperli, O., Achenbach, S., Agewall, S., Badimon, L., Baigent, C., Bueno, H., Bugiardini, R., Carerj, S., Casselman, F., Cuisset, T., Erol, C., Fitzsimons, D., Halle, M., Hamm, C., Hildick-Smith, D., Huber, K., Iliodromitis, E., James, S., Lewis, B. S., Lip, G. Y., Piepoli, M. F., Richter, D., Rosemann, T., Sechtem, U., Steg, P. G., Vrints, C. and Luis Zamorano, J. (2016) '2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC).' *Eur Heart J*, 37(3), Jan 14, 2015/09/01, pp. 267-315.

Rosalki, S. B., Roberts, R., Katus, H. A., Giannitsis, E., Ladenson, J. H. and Apple, F. S. (2004) 'Cardiac biomarkers for detection of myocardial infarction: perspectives from past to present.' *Clin Chem*, 50(11), Nov, pp. 2205-2213.

Ruff, C. T., Bonaca, M. P., Kosowsky, J. M., Conrad, M. J., Murphy, S. A., Jarolim, P., Donahoe, S. M., O'Donoghue, M. L. and Morrow, D. A. (2013) 'Evaluation of the diagnostic performance of heart-type fatty acid-binding protein in the BWH-TIMI ED chest pain study.' *J Thromb Thrombolysis*, 36

Ryan, R. J., Lindsell, C. J., Hollander, J. E., O'Neil, B., Jackson, R., Schreiber, D., Christenson, R. and Gibler, W. B. (2009) 'A Multicenter Randomized Controlled Trial Comparing Central Laboratory and Point-of-Care Cardiac Marker Testing Strategies: The Disposition Impacted by Serial Point of Care Markers in Acute Coronary Syndromes (DISPO-ACS) Trial.' *Annals of Emergency Medicine*, 53(3), Mar, pp. 321-328.

Sardi, A. R., Lamoureux, J. A., Cohn, T. M. and Phillip-Samuel, S. G. (2016) 'Point-of-Care Testing of Troponin Levels Compared With Automated Laboratory Evaluation: A Reliability Study.' *Critical Care Nursing Quarterly*, 39(4), Oct-Dec, pp. 345-351.

Sato, Y., Fujiwara, H. and Takatsu, Y. (2012) 'Cardiac troponin and heart failure in the era of high-sensitivity assays.' *J Cardiol*, 60(3), Sep, pp. 160-167.

Sauerbrei, W. and Blettner, M. (2009) 'Interpreting Results in 2 × 2 Tables: Part 9 of a Series on Evaluation of Scientific Publications.' *Deutsches Ärzteblatt International*, 106(48), 11/27

Schneider, H. G., Ablitt, P. and Taylor, J. (2013) 'Improved sensitivity of point of care troponin I values using reporting to below the 99 th percentile of normals.' *Clin Biochem*, 46(12), Aug, 2013/05/01, pp. 979-982.

Scirica, B. M., Cannon, C. P., Antman, E. M., Murphy, S. A., Morrow, D. A., Sabatine, M. S., McCabe, C. H., Gibson, C. M. and Braunwald, E. (2002) 'Validation of the thrombolysis in myocardial infarction (TIMI) risk score for unstable angina pectoris and non-ST-elevation myocardial infarction in the TIMI III registry.' *Am J Cardiol*, 90(3), Aug 01, 2002/07/20, pp. 303-305.

Shah, A. S. V., Anand, A., Sandoval, Y., Lee, K. K., Smith, S. W., Adamson, P. D., Chapman, A. R., Langdon, T., Sandeman, D., Vaswani, A., Strachan, F. E., Ferry, A., Stirzaker, A. G., Reid, A., Gray, A. J., Collinson, P. O., McAllister, D. A., Apple, F. S., Newby, D. E., Mills, N. L. and Investigators, H.-S. (2015) 'High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study.' *Lancet*, 386(10012), Dec 19, pp. 2481-2488.

Sherwood, M. W. and Kristin Newby, L. (2014) 'High-sensitivity troponin assays: evidence, indications, and reasonable use.' *J Am Heart Assoc*, 3(1), Jan 27, 2014/01/29, p. e000403.

Siri-Tarino, P. W., Sun, Q., Hu, F. B. and Krauss, R. M. (2010) 'Saturated fat, carbohydrate, and cardiovascular disease.' *The American Journal of Clinical Nutrition*, 91(3), 01/20

Six, A. J., Backus, B. E. and Kelder, J. C. (2008) 'Chest pain in the emergency room: value of the HEART score.' *Netherlands Heart Journal*, 16(6), Jun, pp. 191-196.

Six, A. J., Backus, B. E., Kingma, A. and Kaandorp, S. I. (2012) 'Consumption of diagnostic procedures and other cardiology care in chest pain patients after presentation at the emergency department.' *Netherlands Heart Journal*, 20(12), Dec, pp. 499-504.

Six, A. J., Cullen, L., Backus, B. E., Greenslade, J., Parsonage, W., Aldous, S., Doevendans, P. A. and Than, M. (2013) 'The HEART score for the assessment of patients with chest pain in the emergency department: a multinational validation study.' *Crit Pathw Cardiol*, 12(3), Sep, pp. 121-126.

Slagman, A., von Recum, J., Möckel, M., Holert, F., Meyer zum Büschenfelde, D., Müller, C. and Searle, J. (2017) 'Diagnostic performance of a high-sensitive troponin T assay and a troponin T point of care assay in the clinical routine of an Emergency Department: A clinical cohort study.' *International Journal of Cardiology*, 230, 3/1/, pp. 454-460.

Slot, M. H. E. B., Rutten, F. H., van der Heijden, G. J. M. G., Doevendans, P. A., Mast, E. G., Bredero, A. C., van der Spoel, O. P., Glatz, J. F. C. and Hoes, A. W. (2013) 'Diagnostic value of a heart-type fatty acid-binding protein (H-FABP) bedside test in suspected acute coronary syndrome in primary care.' *International Journal of Cardiology*, 168(2), Sep 30, pp. 1485-1489.

Smith, J. N., Negrelli, J. M., Manek, M. B., Hawes, E. M. and Viera, A. J. (2015) 'Diagnosis and management of acute coronary syndrome: an evidence-based update.' *J Am Board Fam Med*, 28(2), Mar-Apr, pp. 283-293.

Souter, K. M. (2014) 'Understanding and Dealing With Heart Disease.'

Steg, P. G., James, S. K., Atar, D., Badano, L. P., Blomstrom-Lundqvist, C., Borger, M. A., Di Mario, C., Dickstein, K., Ducrocq, G., Fernandez-Aviles, F., Gershlick, A. H., Giannuzzi, P., Halvorsen, S., Huber, K., Juni, P., Kastrati, A., Knuuti, J., Lenzen, M. J., Mahaffey, K. W., Valgimigli, M., van 't Hof, A.,

Widimsky, P. and Zahger, D. (2012) 'ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation.' *Eur Heart J*, 33(20), Oct, 2012/08/28, pp. 2569-2619.

Storrow, A. B., Zhou, C., Gaddis, G., Han, J. H., Miller, K., Klubert, D., Laidig, A. and Aronsky, D. (2008) 'Decreasing Lab Turnaround Time Improves Emergency Department Throughput and Decreases Emergency Medical Services Diversion: A Simulation Model.' *Academic Emergency Medicine*, 15(11), Nov, pp. 1130-1135.

Strimbu, K. and Tavel, J. A. (2010) 'What are Biomarkers?' *Current opinion in HIV and AIDS*, 5(6) pp. 463-466.

Suetomi, K. and Takahama, K. (1995) 'A sandwich enzyme immunoassay for cardiac troponin I.' *Nihon Hoigaku Zasshi*, 49(1), Feb, 1995/02/01, pp. 26-32.

Suter, R. E. (2007) 'The risk of missed diagnosis of acute myocardial infarction associated with emergency department volume.' *Ann Emerg Med*, 50(2), Aug, pp. 203; author reply 203-204.

Takeda, S., Yamashita, A., Maeda, K. and Maeda, Y. (2003) 'Structure of the core domain of human cardiac troponin in the Ca²⁺-saturated form.' *Nature*, 424(6944), Jul 3, pp. 35-41.

Tanindi, A. and Cemri, M. (2011) 'Troponin elevation in conditions other than acute coronary syndromes.' *Vascular Health and Risk Management*, 7, 09/22 09/21/received, pp. 597-603.

Taylor, C., Forrest-Hay, A. and Meek, S. (2002) 'ROMEO: a rapid rule out strategy for low risk chest pain. Does it work in a UK emergency department?' *Emergency Medicine Journal : EMJ*, 19(5) pp. 395-399.

Ter Avest, E., Visser, A., Reitsma, B., Breedveld, R. and Wolthuis, A. (2016) 'Point-of-care troponin T is inferior to high-sensitivity troponin T for ruling out acute myocardial infarction in the emergency department.' *Eur J Emerg Med*, 23(2), Apr, 2014/12/24, pp. 95-101.

Than, M., Herbert, M., Flaws, D., Cullen, L., Hess, E., Hollander, J. E., Diercks, D., Ardagh, M. W., Kline, J. A., Munro, Z. and Jaffe, A. (2013) 'What is an acceptable risk of major adverse cardiac event in chest pain patients soon after discharge from the Emergency Department?: A clinical survey.' *International Journal of Cardiology*, 166(3), 2013/07/01/, pp. 752-754.

Than, M., Aldous, S., Lord, S. J., Goodacre, S., Frampton, C. M., Troughton, R., George, P., Florkowski, C. M., Ardagh, M., Smyth, D., Jardine, D. L., Peacock, W. F., Young, J., Hamilton, G., Deely, J. M., Cullen, L. and Richards, A. M. (2014a) 'A 2-hour diagnostic protocol for possible cardiac chest pain in the emergency department: a randomized clinical trial.' *JAMA Intern Med*, 174(1), Jan, pp. 51-58.

Than, M., Cullen, L., Aldous, S., Parsonage, W. A., Reid, C. M., Greenslade, J., Flaws, D., Hammett, C. J., Beam, D. M., Ardagh, M. W., Troughton, R., Brown, A. F., George, P., Florkowski, C. M., Kline, J. A., Peacock, W. F., Maisel, A. S., Lim, S. H., Lamanna, A. and Richards, A. M. (2012) '2-Hour accelerated diagnostic protocol to assess patients with chest pain symptoms using contemporary troponins as the only biomarker: the ADAPT trial.' *J Am Coll Cardiol*, 59(23), Jun 05, 2012/05/15, pp. 2091-2098.

Than, M., Flaws, D., Sanders, S., Doust, J., Glasziou, P., Kline, J., Aldous, S., Troughton, R., Reid, C., Parsonage, W. A., Frampton, C., Greenslade, J. H., Deely, J. M., Hess, E., Bin Sadiq, A., Singleton, R., Shopland, R., Vercoe, L., Woolhouse-Williams, M., Ardagh, M., Bossuyt, P., Bannister, L. and Cullen, L. (2014b) 'Development and validation of the Emergency Department Assessment of Chest pain Score and 2 h accelerated diagnostic protocol.' *Emergency Medicine Australasia*, 26(1), Feb, pp. 34-44.

Than, M., Cullen, L., Reid, C. M., Lim, S. H., Aldous, S., Ardagh, M. W., Peacock, W. F., Parsonage, W. A., Ho, H. F., Ko, H. F., Kasliwal, R. R., Bansal, M., Soerianata, S., Hu, D. Y., Ding, R. J., Hua, Q., Seok-Min, K., Sritara, P., Sae-Lee, R., Chiu, T. F., Tsai, K. C., Chu, F. Y., Chen, W. K., Chang, W. H., Flaws, D. F., George, P. M. and Richards, A. M. (2011a) 'A 2-h diagnostic protocol to assess patients with chest pain symptoms in the Asia-Pacific region (ASPECT): a prospective observational validation study.' *Lancet*, 377(9771), Mar-Apr, pp. 1077-1084.

Than, M., Cullen, L., Reid, C. M., Lim, S. H., Aldous, S., Ardagh, M. W., Peacock, W. F., Parsonage, W. A., Ho, H. F., Ko, H. F., Kasliwal, R. R., Bansal, M., Soerianata, S., Hu, D., Ding, R., Hua, Q., Seok-Min, K., Sritara, P., Sae-Lee, R., Chiu, T., Tsai, K. C., Chu, F. Y., Chen, W. K., Chang, W. H., Flaws, D. F., George, P. M. and Richards, A. M. (2011b) 'A 2-h diagnostic protocol to assess patients with chest pain symptoms in the Asia-Pacific region (ASPECT): a prospective observational validation study.' *Lancet*, 377

Thokala, P., Goodacre, S. W., Collinson, P. O., Stevens, J. W., Mills, N. L., Newby, D. E., Morris, F., Kendall, J. and Stevenson, M. D. (2012) 'Cost-effectiveness of presentation versus delayed troponin testing for acute myocardial infarction.' *Heart*, 98(20), Oct, pp. 1498-1503.

Thygesen, K., Alpert, J. S., White, H. D. and Joint, E. S. C. A. A. H. A. W. H. F. T. F. f. t. R. o. M. I. (2007) 'Universal definition of myocardial infarction.' *Eur Heart J*, 28(20), Oct, pp. 2525-2538.

Thygesen, K., Alpert, J. S., Jaffe, A. S., Simoons, M. L., Chaitman, B. R., White, H. D. and AHA, W. G. R. E. A. (2012a) 'The third universal definition of myocardial infarction.' *Kardiologia Polska*, 70, Nov, pp. S235-S254.

Thygesen, K., Alpert, J. S., Jaffe, A. S., Simoons, M. L., Chaitman, B. R., White, H. D. and Force, J. E. A. A. W. T. (2013) 'Third universal definition of myocardial infarction.' *Revista Espanola De Cardiologia*, 66(2), Feb, pp. 2551-2567.

Thygesen, K., Mair, J., Katus, H., Plebani, M., Venge, P., Collinson, P., Lindahl, B., Giannitsis, E., Hasin, Y., Galvani, M., Tubaro, M., Alpert, J. S., Biasucci, L. M., Koenig, W., Mueller, C., Huber, K., Hamm, C., Jaffe, A. S. and ES, S. G. B. C. (2010) 'Recommendations for the use of cardiac troponin measurement in acute cardiac care(dagger).' *European Heart Journal*, 31(18), Sep, pp. 2197-2204b.

Thygesen, K., Alpert, J. S., Jaffe, A. S., Simoons, M. L., Chaitman, B. R., White, H. D., Katus, H. A., Apple, F. S., Lindahl, B., Morrow, D. A., Chaitman, B. R., Clemmensen, P. M., Johanson, P., Hod, H., Underwood, R., Bax, J. J., Bonow, J. J., Pinto, F., Gibbons, R. J., Fox, K. A., Atar, D., Newby, L. K., Galvani, M., Hamm, C. W., Uretsky, B. F., Steg, P. G., Wijns, W., Bassand, J. P., Menasche, P., Ravkilde, J., Ohman, E. M., Antman, E. M., Wallentin, L. C., Armstrong, P. W., Simoons, M. L., Januzzi, J. L., Nieminen, M. S., Gheorghiade, M., Filippatos, G., Luepker, R. V., Fortmann, S. P., Rosamond, W. D., Levy, D., Wood, D., Smith, S. C., Hu, D., Lopez-Sendon, J. L., Robertson, R. M., Weaver, D., Tendera, M., Bove, A. A., Parkhomenko, A. N., Vasilieva, E. J., Mendis, S., Bax, J. J., Baumgartner, H., Ceconi, C., Dean, V., Deaton, C., Fagard, R., Funck-Brentano, C., Hasdai, D., Hoes, A., Kirchhof, P., Knuuti, J., Kolh, P., McDonagh, T., Moulin, C., Popescu, B. A., Reiner, Z., Sechtem, U., Sirnes, P. A., Tendera, M., A, T., Vahanian, A., Windecker, S., Morais, J., Aguiar, C., Almahmeed, W., Arnar, D. O., Barili, F., Bloch, K. D., Bolger, A. F., Botker, H. E., Bozkurt, B., Bugiardini, R., Cannon, C., de Lemos, J., Eberli, F. R., Escobar, E., Hlatky, M., James, S., Kern, K. B., Moliterno, D. J., Mueller, C., Neskovic, A. N., Pieske, B. M., Schulman, S. P., Storey, R. F., Taubert, K. A., Vranckx, P., Wagner, D. R. and Force, J. E. A. A. W. T. (2012b) 'Third universal definition of myocardial infarction.' *Nature Reviews Cardiology*, 9(11), Nov, pp. 620-633.

Timmis, A. (2015) 'Acute coronary syndromes.' *BMJ : British Medical Journal*, 351

Torio, C. M. and Andrews, R. M. (2006) 'National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2011: Statistical Brief #160.' *In Healthcare Cost and Utilization Project (HCUP) Statistical Briefs*. Rockville MD

Tortora, G. J. and Grabowski, S. R. (1996) *Principle of human anatomy and physiology*. HarperCollins.

'Troponins, Cardiac A2 - Wilson, David A.' (2012) *In Clinical Veterinary Advisor*. Saint Louis: W.B. Saunders, pp. 966-967.

Va Den Berg, P., Burrows, G., Lewis, P., Carley, S. and Body, R. (2017) 'Validation of the (Troponin-only) Manchester Acute Coronary Syndromes decision aid with a contemporary cardiac troponin I assay.' *Am J Emerg Med*, Sep 23,

Valle, H. A., Riesgo, L. G., Bel, M. S., Gonzalo, F. E., Sanchez, M. S. and Oliva, L. I. (2008) 'Clinical assessment of heart-type fatty acid binding protein in early diagnosis of acute coronary syndrome.' *Eur J Emerg Med*, 15(3), Jun, 2008/05/08, pp. 140-144.

Van Den Berg, P. and Body, R. (2017) 'The HEART score for early rule out of acute coronary syndromes in the emergency department: a systematic review and meta-analysis.' *Eur Heart J Acute Cardiovasc Care*, May 01, p. 2048872617710788.

VanNieuwenhoven, F. A., Musters, R. J. P., Post, J. A., Verkleij, A. J., VanderVusse, G. J. and Glatz, J. F. C. (1996) 'Release of proteins from isolated neonatal rat cardiomyocytes subjected to simulated ischemia or metabolic inhibition is independent of molecular mass.' *Journal of Molecular and Cellular Cardiology*, 28(7), Jul, pp. 1429-1434.

Varki, A. P., Roby, D. S., Watts, H. and Zatuchni, J. (1978) 'Serum myoglobin in acute myocardial infarction: a clinical study and review of the literature.' *Am Heart J*, 96(5), Nov, 1978/11/01, pp. 680-688.

Venge, P., Ohberg, C., Flodin, M. and Lindahl, B. (2010) 'Early and late outcome prediction of death in the emergency room setting by point-of-care and laboratory assays of cardiac troponin I.' *American Heart Journal*, 160(5), Nov, pp. 835-841.

Venge, P., van Lippen, L., Blaschke, S., Christ, M., Geier, F., Giannitsis, E., Hagström, E., Hausfater, P., Khellaf, M., Mair, J., Pariente, D., Scharnhorst, V. and Semjonow, V. (2017) 'Equal clinical performance of a novel point-of-care cardiac troponin I (cTnI) assay with a commonly used high-sensitivity cTnI assay.' *Clinica Chimica Acta*, 469, 6//, pp. 119-125.

Verbrugge, F. H., Kellens, S., Vanmechelen, M., Grieten, L., Van Lierde, J., Dens, J., Vrolix, M. and Vandervoort, P. (2015) 'Point-of-care heart-type fatty acid binding protein versus high-sensitivity troponin T testing in emergency patients at high risk for acute coronary syndrome.' *European Heart Journal*, 36, Aug 1, pp. 8-8.

Viera, A. J. and Garrett, J. M. (2005) 'Understanding interobserver agreement: the kappa statistic.' *Family Medicine*, 37(5), May, pp. 360-363.

Vile, J. L., Allkins, E., Frankish, J., Garland, S., Mizen, P. and Williams, J. E. (2017) 'Modelling patient flow in an emergency department to better understand demand management strategies.' *Journal of Simulation*, 11(2), 2017/05/01, pp. 115-127.

Visser, A., Wolthuis, A., Breedveld, R. and ter Avest, E. (2014) 'HEART score and clinical gestalt have similar diagnostic accuracy for diagnosing ACS in an unselected population of patients with chest pain presenting in the ED.' *Emergency Medicine Journal*,

Walter, S. D., Eliasziw, M. and Donner, A. (1998) 'Sample size and optimal designs for reliability studies.' *Statistics in Medicine*, 17(1), Jan 15, pp. 101-110.

Wang, J., Tan, G.-J., Han, L.-N., Bai, Y.-Y., He, M. and Liu, H.-B. (2017) 'Novel biomarkers for cardiovascular risk prediction.' *Journal of Geriatric Cardiology : JGC*, 14(2), 12/09/received, pp. 135-150.

Wang, J. C., Normand, S. L. T., Mauri, L. and Kuntz, R. E. (2004) 'Coronary artery spatial distribution of acute myocardial infarction occlusions.' *Circulation*, 110(3), Jul 20, pp. 278-284.

Weigl, B. H., Gaydos, C. A., Kost, G., Beyette, F. R., Jr., Sabourin, S., Rompalo, A., de Los Santos, T., McMullan, J. T. and Haller, J. (2012) 'The Value of Clinical Needs Assessments for Point-of-Care Diagnostics.' *Point Care*, 11(2), Jun, pp. 108-113.

Weissberg, P. L. (1999) 'Atherosclerosis involves more than just lipids: plaque dynamics.' *European Heart Journal Supplements*, 1(T), Dec, pp. T13-T18.

WHO. (1979) 'Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature.' *Circulation*, 59(3) pp. 607-609.

Willemsen, R. T. A., Dinant, G. J. and Glatz, J. F. C. (2015a) 'Biomarkers of Myocardial Cell Damage: Heart-Type Fatty Acid Binding Protein (H-FABP) for the Early Evaluation of Suspected Acute Coronary Syndrome.' *In* Patel, V. B. and Preedy, V. R. (eds.) *Biomarkers in Cardiovascular Disease*. Dordrecht: Springer Netherlands, pp. 1-31.

Willemsen, R. T. A., Dinant, G. J. and Glatz, J. F. C. (2016) 'Biomarkers of Myocardial Cell Damage: Heart-Type Fatty Acid Binding Protein (H-FABP) for the Early Evaluation of Suspected Acute Coronary Syndrome.'

Willemsen, R. T. A., van Severen, E., Vandervoort, P. M., Grieten, L., Buntinx, F., Glatz, J. F. C. and Dinant, G. J. (2015b) 'Heart-type fatty acid binding protein (H-FABP) in patients in an emergency department setting, suspected of acute coronary syndrome: Optimal cut-off point, diagnostic value and future opportunities in primary care.' *European Journal of General Practice*, 21(3), 2015/07/03, pp. 156-163.

Wodzig, K. W. H., Pelsers, M. M. A. L., vanderVusse, G. J., Roos, W. and Glatz, J. F. C. (1997) 'One-step enzyme-linked immunosorbent assay (ELISA) for plasma fatty acid-binding protein.' *Annals of Clinical Biochemistry*, 34, May, pp. 263-268.

Wodzig, K. W. H., Kragten, J. A., Modrzejewski, W., Górski, J., van Dieijen-Visser, M. P., Glatz, J. F. C. and Hermens, W. T. (1998) 'Thrombolytic therapy does not change the release ratios of enzymatic and non-enzymatic myocardial marker proteins.' *Clinica Chimica Acta*, 272(2), 1998/04/27/, pp. 209-223.

World Health, O. (2014) *World health statistics 2014*. Geneve: World Health Organization.

Wu, A. H. (1999) 'Biochemical markers of cardiac damage: from traditional enzymes to cardiac-specific proteins. IFCC Subcommittee on Standardization of Cardiac Markers (S-SCM).' *Scand J Clin Lab Invest Suppl*, 230 pp. 74-82.

Wu, A. H. B. (2017) 'Release of cardiac troponin from healthy and damaged myocardium.' *Frontiers in Laboratory Medicine*, 2017/11/03/,

Xu, L.-Q., Yang, Y.-M., Tong, H. and Xu, C.-F. (2017) 'Early Diagnostic Performance of Heart-Type Fatty Acid Binding Protein in Suspected Acute Myocardial Infarction: Evidence From a Meta-Analysis of Contemporary Studies.' *Heart, Lung and Circulation*, 2017/05/04/,

Yarmohammadian, M. H., Rezaei, F., Haghshenas, A. and Tavakoli, N. (2017) 'Overcrowding in emergency departments: A review of strategies to decrease future challenges.' *Journal of Research in Medical Sciences*, 22, Feb,

Yolken, R. H. (1982) 'Enzyme Immunoassays for the Detection of Infectious Antigens in Body-Fluids - Current Limitations and Future-Prospects.' *Reviews of Infectious Diseases*, 4(1) pp. 35-68.

Zaninotto, M., Mion, M., Altinier, S., Forni, M. and Plebani, M. (2004) 'Quality specifications for biochemical markers of myocardial injury.' *Clinica Chimica Acta*, 346(1), Aug, pp. 65-72.

Huang, J. X. and Ma, C. S. (2010) 'Effect of cigarette smoking on clinical outcomes of hospitalized Chinese male smokers with acute myocardial infarction.' *Chinese Medical Journal*, 123(20), Oct 20, pp. 2807-2811.

Zoltani, C. K. (2014) 'Chapter 11 - Cardiovascular toxicity biomarkers A2 - Gupta, Ramesh C.' *In Biomarkers in Toxicology*. Boston: Academic Press, pp. 199-215.

Wang, J. C., Normand, S. L. T., Mauri, L. and Kuntz, R. E. (2004) 'Coronary artery spatial distribution of acute myocardial infarction occlusions.' *Circulation*, 110(3), Jul 20, pp. 278-284.

Weigl, B. H., Gaydos, C. A., Kost, G., Beyette, F. R., Jr., Sabourin, S., Rompalo, A., de Los Santos, T., McMullan, J. T. and Haller, J. (2012) 'The Value of Clinical Needs Assessments for Point-of-Care Diagnostics.' *Point Care*, 11(2), Jun, pp. 108-113.

Weissberg, P. L. (1999) 'Atherosclerosis involves more than just lipids: plaque dynamics.' *European Heart Journal Supplements*, 1(T), Dec, pp. T13-T18.

WHO. (1979) 'Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature.' *Circulation*, 59(3) pp. 607-609.

Willemsen, R. T. A., Dinant, G. J. and Glatz, J. F. C. (2015a) 'Biomarkers of Myocardial Cell Damage: Heart-Type Fatty Acid Binding Protein (H-FABP) for the Early Evaluation of Suspected Acute Coronary Syndrome.' *In Patel, V. B. and Preedy, V. R. (eds.) Biomarkers in Cardiovascular Disease*. Dordrecht: Springer Netherlands, pp. 1-31.

Willemsen, R. T. A., Dinant, G. J. and Glatz, J. F. C. (2016) 'Biomarkers of Myocardial Cell Damage: Heart-Type Fatty Acid Binding Protein (H-FABP) for the Early Evaluation of Suspected Acute Coronary Syndrome.'

Willemsen, R. T. A., van Severen, E., Vandervoort, P. M., Grieten, L., Buntinx, F., Glatz, J. F. C. and Dinant, G. J. (2015b) 'Heart-type fatty acid binding protein (H-FABP) in patients in an emergency department setting, suspected of acute coronary syndrome: Optimal cut-off point, diagnostic value and future opportunities in primary care.' *European Journal of General Practice*, 21(3), 2015/07/03, pp. 156-163.

Wodzig, K. W. H., Pelsers, M. M. A. L., vanderVusse, G. J., Roos, W. and Glatz, J. F. C. (1997) 'One-step enzyme-linked immunosorbent assay (ELISA) for plasma fatty acid-binding protein.' *Annals of Clinical Biochemistry*, 34, May, pp. 263-268.

Wodzig, K. W. H., Kragten, J. A., Modrzejewski, W., Górski, J., van Dieijen-Visser, M. P., Glatz, J. F. C. and Hermens, W. T. (1998) 'Thrombolytic therapy does not change the release ratios of enzymatic and non-enzymatic myocardial marker proteins.' *Clinica Chimica Acta*, 272(2), 1998/04/27/, pp. 209-223.

World Health, O. (2014) *World health statistics 2014*. Geneve: World Health Organization.

Wu, A. H. (1999) 'Biochemical markers of cardiac damage: from traditional enzymes to cardiac-specific proteins. IFCC Subcommittee on Standardization of Cardiac Markers (S-SCM).' *Scand J Clin Lab Invest Suppl*, 230 pp. 74-82.

Wu, A. H. B. (2017) 'Release of cardiac troponin from healthy and damaged myocardium.' *Frontiers in Laboratory Medicine*, 2017/11/03/,

Xu, L.-Q., Yang, Y.-M., Tong, H. and Xu, C.-F. (2017) 'Early Diagnostic Performance of Heart-Type Fatty Acid Binding Protein in Suspected Acute Myocardial Infarction: Evidence From a Meta-Analysis of Contemporary Studies.' *Heart, Lung and Circulation*, 2017/05/04/,

Yarmohammadian, M. H., Rezaei, F., Haghshenas, A. and Tavakoli, N. (2017) 'Overcrowding in emergency departments: A review of strategies to decrease future challenges.' *Journal of Research in Medical Sciences*, 22, Feb,

Yolken, R. H. (1982) 'Enzyme Immunoassays for the Detection of Infectious Antigens in Body-Fluids - Current Limitations and Future-Prospects.' *Reviews of Infectious Diseases*, 4(1) pp. 35-68.

Zaninotto, M., Mion, M., Altinier, S., Forni, M. and Plebani, M. (2004) 'Quality specifications for biochemical markers of myocardial injury.' *Clinica Chimica Acta*, 346(1), Aug, pp. 65-72.

Zhang, H., Sun, S. A., Tong, L., Li, R., Cao, X. H., Zhang, B. H., Zhang, L. H., Huang, J. X. and Ma, C. S. (2010) 'Effect of cigarette smoking on clinical outcomes of hospitalized Chinese male smokers with acute myocardial infarction.' *Chinese Medical Journal*, 123(20), Oct 20, pp. 2807-2811.

Zoltani, C. K. (2014) 'Chapter 11 - Cardiovascular toxicity biomarkers A2 - Gupta, Ramesh C.' *In Biomarkers in Toxicology*. Boston: Academic Press, pp. 199-215.

Appendices

Appendix 1.1

Study	Protocol	POC device	Analytical characteristic	Reference standards	timing of troponin measurement	cTn decision limit	Outcome AMI (%)	NPV	Sensitivity	specificity	PPV
Than et al 2012 ADP protocol (ASPECT)[1]	TIMI, ECG, POCT cardiac panel, pre-test probability	The Alere Triage® Troponin I	LoB 0.05	High sensitivity assay.	at presentation and 2h afterward	.05 µg/L	349 (9.7%)	96.1%	82.9%	56.0%	20.1%
al dous et al 2012 [2]	ADP protocol (TIMI, ECG, POCT cardiac panel, pre-test probability)	The Alere Triage® Troponin I	LoB 0.05	ARCHITECT STAT assay con	at presentation and 2h afterward	.05 µg/L	(15.3%) BY ADP using Torp POC	88.7%	62.0 %	95.6%	82.0%
al dous et al 2014 [3]	ADP protocol (TIMI, ECG, POCT cardiac panel, pre-test probability) and Architect cTnI 28 ng/L AT Time 0 & Time 6 - 12 CARDIO TO vs T2'	Alere cardio 3 POC cTnI	99th Percentile Troponin I 0.02 ng/mL, Analytical Sensitivity Troponin I LoB <0.01 ng/mL	Architect cTnI 28 ng/L AT Time 0 & Time 6 - 12	at presentation and 2h afterward	99th per= 20 ng/L	220 (22.9%) had AMI (196 NSTEMI)	T0 96.2%	T0 87.8% T2 93.6	T0 93.1% T2 90.2	79.1 %
Diercks et al 2012 [4] MIDAS Study	cTnI AND cTnT Assays with varying local decision limits AHA/ACC criteria for AMI	Alere cardio 3 POC cTnI	<0.01 ng/mL the limit of detection	Beckman Dxl AccuTnI Definition ACC/ESC	at presentation and 90 min, 3 hours, 6 hours	99th per= .05 µg/L	10 % AMI diagnosed in 82 patients	T0 96.0% T3 98.1%	T0 66.7% , T3 84.7	T0 95.9% T3 93.4%	T- 65.8% T3 60.4 %
Lee - lewandro et al 2011 [5]	The study copare 2 commonly used POC Strategies to fourth generation central lab assay on first draw	i-STAT POC and Biosite Triage cardiac reader		Elecsys E10 Forth generation cardiac troponin T assay	at presentation	cTnI i-STAT 0.08 ,Biosite Triage cardiac instrument (old Alere) 99th Percentile Troponin I 0.05 , MMP 0.39 and cTnT Roch <0.03	11% AMI diagnosed in 22 patients	95% for cTnI i-STAT	63% for cTnI i-STAT	94% for cTnI i-STAT	58% for cTnI i-STAT
Stengaard , C et al 2013 [6]	AMI was diagnosed according to the universal definition of AMI using the 99th percentile upper reference level as diagnostic cut point. Paramedics record ECG and symptoms	Roche cobas h232 cTnT	LoB :0.1 µg/L (ng/mL) LoD : 0.16 µg/L (ng/mL)	high sensitivity troponin assay Roche reference level (14 ng/L)	sample obtained by paramedics before 88 min of routine sample in the hospital	cTnT >50 ng/L		86%	39%	95%	68%
Study	Protocol	POC device	Analytical characteristic	Reference standards	timing of troponin measurement	cTn decision limit	Outcome AMI (%)	NPV	Sensitivity	specificity	PPV
palamalai et al 2013 [7]	AMI was diagnosed according to 2007 universal definition of MI clinical symptoms and cTn > 99th	(PATHFAST) cTnI	(PATHFAST) LoD 8 ng/L, 99th percentile	Vitros ECI ES	at presentation, 3 hours post presentation and 6 hours post presentation	29 ng/L, %CV at 99th percentile 5%.	11.2% AMI detected	0h: 93% 3h: 98% 6h: 99% Max: 99%	0h: 53% 3h: 89% 6h: 95% Max: 95%	0h: 86% 3h: 82% 6h: 82% Max: 78%	0h: 32% 3h: 39% 6h: 40% Max: 35%

	percentile/Vitros cTnI)		29 ng/L, %CV at 99th percentile 5%.								
palamalai et al 2013 [7]	AMI was diagnosed according to 2007 universal definition of MI clinical symptoms and cTn > 99th percentile (Vitros cTnI)	Radiometer AQT90 (POC)	(AQT90) LoD 9 ng/L, 99th percentile 23 ng/L, %CV at 99th percentile 17%	Vitros ECI ES (Ortho-Clinical Diagnostics, Rochester NY)	at presentation, 3 hours post presentation and 6 hours post presentation	99th percentile 23 ng/L, %CV at 99th percentile 17%	11.2% AMI detected	0h: 91% 3h: 95% 6h: 95% Max: 95%	0h: 26% 3h: 63% 6h: 63% Max: 68%	0h: 93% 3h: 91% 6h: 91% Max: 89%	0h: 31% 3h: 48% 6h: 46% Max: 43%
palamalai et al 2013 [7]	AMI was diagnosed according to 2007 universal definition of MI clinical symptoms and cTn > 99th percentile (Vitros cTnI)	Abbott i-STAT (POC) cTnI	LoD 20 ng/L, 99th percentile 80 ng/L, %CV at 99th percentile 16%	Vitros ECI ES (Ortho-Clinical Diagnostics, Rochester NY)	at presentation, 3 hours post presentation and 6 hours post presentation	LoD 20 ng/L, 99th percentile 80 ng/L, %CV at 99th percentile 16%	11.2% AMI detected	0h: 91% 3h: 96% 6h: 96% Max: 96%	0h: 32% 3h: 68% 6h: 95% Max: 95%	0h: 92% 3h: 90% 6h: 91% Max: 88%	0h: 33% 3h: 46% 6h: 50% Max: 44%
palamalai et al 2013 [7]	AMI was diagnosed according to 2007 universal definition of MI clinical symptoms and cTn > 99th percentile (Vitros cTnI)	Gem Immune (Instrumentation Laboratory, Bedford MA)	(IL GEM) LoD 1.3 ng/L, 99th percentile 15 ng/L, %CV at 99th percentile 14%	Vitros ECI ES (Ortho-Clinical Diagnostics, Rochester NY)	at presentation, 3 hours post presentation and 6 hours post presentation	(IL GEM) LoD 1.3 ng/L, 99th percentile 15 ng/L, %CV at 99th percentile 14%	11.2% AMI detected	0h: 95% 3h: 98% 6h: 99% Max: 99%	0h: 63% 3h: 89% 6h: 95% Max: 95%	0h: 85% 3h: 82% 6h: 80% Max: 79%	0h: 34% 3h: 39% 6h: 38% Max: 36%
Birkhahn et al [10]	2 troponin T (TnT) enzymes obtained 6 hours apart.	Triage MeterPlus CKMB, troponin I (TnI), and myoglobin	The manufacturer of the triage cardiac panel quotes cutoff values for POC TnI, myoglobin, and creatine kinase MB of 0.05 ng/mL (99th percentile; coefficient of variation [CV], 25%), 107 ng/mL (95%; CV, 10%), and 4.3 ng/mL (95%; CV, 10%), respectively	Hitachi Modular (Roche).	at presentation and 2 hours post presentation by POC and T0,T6 central lab test	if POC TnI of 0.1 ng/mL or higher or myoglobin of 150 ng/mL or higher were considered abnormal	12 patients with elevated TnI and/or myoglobin	100%	100%	65%	20%
Study	Protocol	POC device	Analytical characteristic	Reference standards	timing of troponin measurement	cTn decision limit	Outcome AMI (%)	NPV	Sensitivity	specificity	PPV

Andersson, P.O., et al 2015 [12]	The diagnoses of AMI protocol to find acute myocardial infarction (AMI) and the combination of AMI or unstable angina (UA) based on result measured by high sensitivity assay and POC and UA were based on the Third universal definition of myocardial infarction	Cobas h232 (Roche Diagnostics,	The detection limit was 0.03 µg/L (30 ng/L) and all values >0.03 µg/L (>30 ng/L) were regarded as positive according	Cobas e602 instrument (Roche Diagnostics, limit of detection of 1 ng/L. A decision limit of ≥15 ng/L	at presentation	in POC value above >30 ng/L) were regarded as positive and hs-cTnT-T ≥ 15 ng/L	Only three had AMI and three had UA. Thirty-one patients had hs-cTnT >15 ng/L.	POCT-cTnT for AMI 99% hs-cTnT 100% FOR AMI/UA POCT-cTnT 96% hs-cTnT 99%	POCT-cTnT for AMI 67% hs-cTnT 100% FOR AMI/UA POCT-cTnT 33% hs-cTnT 83%	POCT-cTnT for AMI 98% hs-cTnT 75% FOR AMI/UA POCT-cTnT 98% hs-cTnT 76%	POCT-cTnT for AMI 50% hs-cTnT 10% FOR AMI/UA POCT-cTnT 50% hs-cTnT 16%
Planer, D., et al. 2006	Acute evolving or recent myocardial infarction was defined according to the consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction	Roche CARDIAC Trop T Sensitive test					The prevalence of myocardial infarction was 1.7%	100%	100%	100%	100%
Straface, A.L., et al. 2008 [13]	The diagnosis of (AMI) was based on a doubling myoglobin level accompanied by at least a 50% increase in the creatine kinase (CK)-MB level with no detectable TnI; a doubling of myoglobin level together with any detectable TnI; or a TnI level of 0.4 ng/mL	Biosite Triage Cardiac Panel									

Appendix 2.1.

Patient Name: _____ DOB _____ NUMBER _____

CARDIAC CHEST PAIN PATHWAY

DATE/TIME OF ASSESSMENT: _____ DOCTOR: _____ GRADE: _____

SYMPTOMS: Chest pain/discomfort ☐
Cardiac arrest at admission ☐

ONSET

Time of onset: _____ Time of peak symptoms: _____

Duration of symptoms: _____ More than 2 episodes in 24h? Yes ☐ No ☐

Activity at onset: _____ Constant ☐ Intermittent ☐

MAIN SITE OF PAIN

Central chest ☐ Left anterior chest ☐ Left lateral chest ☐ Right anterior chest ☐
Epigastrium ☐ Back ☐ Arms/shoulders ☐ Jaw/neck/throat ☐
Other: _____

PAIN CHARACTER

Heavy ☐ Pressure ☐ Crushing ☐ Tight or squeezing ☐
Sharp/stabbing ☐ Dull ☐ Burning ☐ Indigestion-like ☐
Other: _____

PAIN RADIATION

Left arm ☐ Left shoulder ☐ Right arm ☐ Right shoulder ☐
Throat or jaw ☐ Neck ☐ Epigastrium ☐ Back ☐
Left chest ☐ Right chest ☐ Legs ☐ None ☐

ASSOCIATED SYMPTOMS

Sweating ☐ Nausea ☐ Vomiting ☐ Dyspnoea ☐
Light-headedness ☐ Vertigo ☐ Syncope ☐ Belching ☐
Other: _____ None ☐

EXACERBATING FACTORS

Inspiration ☐ Coughing ☐ Movement ☐ Posture ☐
Exertion ☐ Emotions/stress ☐ Touch/palpation ☐ None ☐
Other, state _____

RELIEVING FACTORS

GTN ☐ Rest ☐ Antacids ☐ None ☐
Other, state _____

Maximum pain score (0, none, to 10, maximum): /10

In my opinion, for an acute coronary syndrome these symptoms are:

Highly suspicious ☐ Moderately suspicious ☐ Slightly suspicious ☐
Is this a pattern of worsening angina? Y ☐ N ☐ Did the pain occur at rest? Y ☐ N ☐
Pain the same as a previous AMI? Y ☐ N ☐ Is the patient still in pain? Y ☐ N ☐

PAST MEDICAL HISTORY

Myocardial infarction Y ☐ N ☐ How many? _____ Date of last MI: _____
Prior angina Y ☐ N ☐ Pacemaker/ICD Y ☐ N ☐
Previous PCI/stent Y ☐ N ☐ Date of last PCI: _____
Previous CABG Y ☐ N ☐ Date of last CABG: _____
Arrhythmia Y ☐ N ☐ Details (if known): _____

Risk factors:

Hypertension Y ☐ N ☐ Hyperlipidaemia Y ☐ N ☐ (Ever had or been treated for?)
Type 1 diabetes Y ☐ N ☐ Type 2 diabetes Y ☐ N ☐

Other past history

Stroke or TIA Y ☐ N ☐ Peripheral arterial disease Y ☐ N ☐ Heart failure Y ☐ N ☐
Renal impairment Y ☐ N ☐ Rheumatoid arthritis Y ☐ N ☐
Others: _____

MEDICATION HISTORY

Case Report Form, Version 1.0. 27 January 2015. Page 1 of 2
Full Study Title: The Bedside Evaluation of Sensitive Troponin (BEST) study

Patient Name: _____ DOB _____ NUMBER _____

Aspirin ☐ Y ☐ N ☐ ACE inhibitor ☐ Y ☐ N ☐ Others: _____

Clopidogrel ☐ Y ☐ N ☐ Beta blocker ☐ Y ☐ N ☐

Prasugrel ☐ Y ☐ N ☐ Ca²⁺ blocker ☐ Y ☐ N ☐

Ticagrelor ☐ Y ☐ N ☐ Insulin ☐ Y ☐ N ☐

Oral nitrate ☐ Y ☐ N ☐ Nicorandil ☐ Y ☐ N ☐

Statin ☐ Y ☐ N ☐ Allergies: _____

RELEVANT SOCIAL HISTORY

Current smoking ☐ Y ☐ N ☐ Smoking in last 4/52 ☐ Y ☐ N ☐ Smoking ever? ☐ Y ☐ N ☐

Alcohol use? ☐ Y ☐ N ☐ More than 21u (men) or 14u (women)? ☐ Y ☐ N ☐

Family history of IHD? ☐ Y ☐ N ☐ (In a 1st degree relative aged <65 years)

ON EXAMINATION

Heart Rate: _____ Respiratory Rate _____ Blood pressure _____ SpO₂ _____ Temp _____

Heart sounds normal? ☐ Y ☐ N ☐ Details: _____

Respiratory examination:

Crepitations at bases? ☐ Y ☐ N ☐ Crepitations to mid-zones or above? ☐ Y ☐ N ☐

Killip Class: 1. No heart failure ☐ 2. Basal crackles or raised JVP ☐

3. Pulmonary oedema ☐ 4. Cardiogenic shock ☐

Other details: _____

Abdominal examination: Abdominal tenderness? ☐ Y ☐ N ☐

Other details: _____

Is the patient visibly sweating? ☐ Y ☐ N ☐

Is the chest wall tender? ☐ Y ☐ N ☐ Does palpation of the chest reproduce the pain? ☐ Y ☐ N ☐

Other: _____

IMPRESSION AND PLAN

Notes:

ECG interpretation:

Acute ischaemia ☐ Y ☐ N ☐ ST elevation (STEMI) ☐ Y ☐ N ☐ LBBB ☐ Y ☐ N ☐

ST depression ☐ Y ☐ N ☐ Abnormal T inversion ☐ Y ☐ N ☐ Dynamic changes? ☐ Y ☐ N ☐

How likely is ACS? (Tick the closest to your estimated probability)

Definite ☐ Probable ☐ Could be ☐ Probably not ☐ Definitely not ☐

Estimated probability of ACS (0-100%): _____

Disposition: After the ED, where is the patient going? (Or where did they go?)

Home ☐ OMU ☐ MAU/medical ward ☐ CCU/Cardiology ☐ Cath Lab ☐

TIME OF DECISION:

Treatment: In the ED, did the patient receive?..

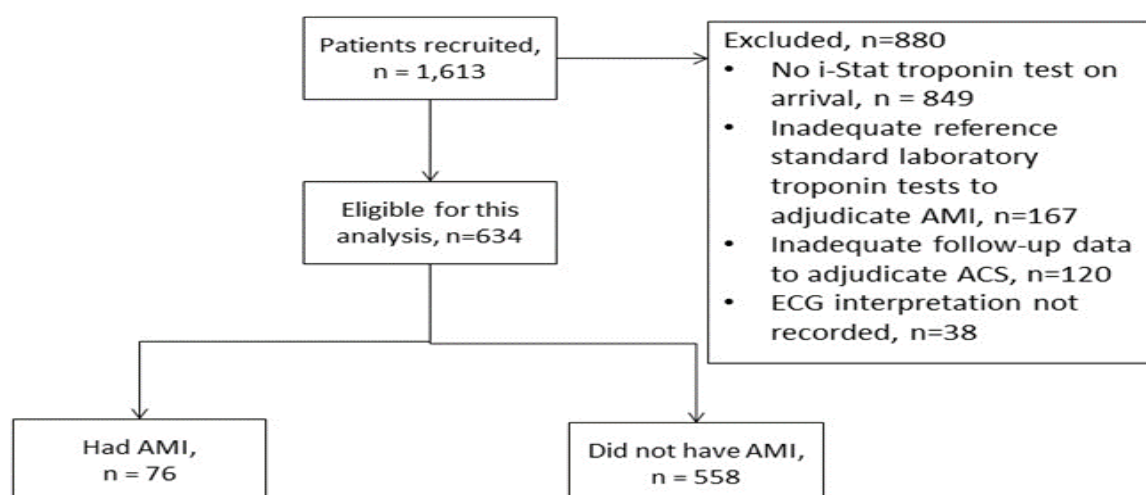
Aspirin ☐ Clopidogrel ☐ Prasugrel ☐ Ticagrelor ☐ Fondaparinux/LMWH ☐

Thrombolysis ☐ Referral for primary PCI ☐ Morphine ☐ GTN ☐ Oxygen ☐

Chest X-ray: Requested? ☐ Y ☐ N ☐ Pulmonary oedema? ☐ Y ☐ N ☐ Upper lobe diversion? ☐ Y ☐ N ☐

Signature: _____ Print: _____

Appendix 2.2



The basic details of each recruiting centre

Site number	Site name	Annual number of ED attendances	Type of centre
1	Central Manchester University Hospitals NHS Foundation Trust	985,29	Teaching hospital
2	Basildon & Thurrock University Hospitals NHS Foundation Trust	115089	District General Hospital
3	Wirral University Teaching Hospital NHS Foundation Trust	89652	Teaching hospital
4	Imperial College Healthcare NHS Trust	273,355	Teaching hospital
5	St. George's Healthcare NHS Trust	154,418	

6	Royal Bolton Hospital NHS Foundation Trust	112994	
7	Taunton and Somerset NHS Foundation Trust	585,70	
8	Royal Devon & Exeter NHS Foundation Trust	105,999	
9	Harrogate and District NHS Foundation Trust	483,18	
10	Northumbria Healthcare NHS Foundation Trust	166,777	
11	Basingstoke and North Hampshire NHS Trust	119190	
12	Nottingham University Hospital NHS Foundation Trust	194371	Teaching hospital
13	University Hospital Birmingham NHS Trust	102,054	Teaching hospital
14	Kings College Hospital NHS Foundation Trust	231578	Teaching hospital

Appendix 2.3

Patient Name: _____ DOB: _____ NUMBER: _____

CARDIAC CHEST PAIN PATHWAY

DATE/TIME OF ASSESSMENT: _____ DOCTOR: _____ GRADE: _____

SYMPTOMS: Chest pain/discomfort ☐
Cardiac arrest at admission ☐

ONSET

Time of onset: _____ Time of peak symptoms: _____
Duration of symptoms: _____ More than 2 episodes in 24h? Yes ☐ No ☐
Activity at onset: _____ Constant ☐ Intermittent ☐

MAIN SITE OF PAIN

Central chest ☐ Left anterior chest ☐ Left lateral chest ☐ Right anterior chest ☐
Epigastrium ☐ Back ☐ Arms/shoulders ☐ Jaw/neck/throat ☐
Other: _____

PAIN CHARACTER

Heavy ☐ Pressure ☐ Crushing ☐ Tight or squeezing ☐
Sharp/stabbing ☐ Dull ☐ Burning ☐ Indigestion-like ☐
Other: _____

PAIN RADIATION

Left arm ☐ Left shoulder ☐ Right arm ☐ Right shoulder ☐
Throat or jaw ☐ Neck ☐ Epigastrium ☐ Back ☐
Left chest ☐ Right chest ☐ Legs ☐ None ☐

ASSOCIATED SYMPTOMS

Sweating ☐ Nausea ☐ Vomiting ☐ Dyspnoea ☐
Light-headedness ☐ Vertigo ☐ Syncope ☐ Belching ☐
Other: _____ None ☐

EXACERBATING FACTORS

Inspiration ☐ Coughing ☐ Movement ☐ Posture ☐
Exertion ☐ Emotions/stress ☐ Touch/palpation ☐ None ☐
Other, state _____

RELIEVING FACTORS

GTN ☐ Rest ☐ Antacids ☐ None ☐
Other, state _____

Maximum pain score (0, none, to 10, maximum): /10

In my opinion, for an acute coronary syndrome these symptoms are:
Highly suspicious ☐ Moderately suspicious ☐ Slightly suspicious ☐
Is this a pattern of worsening angina? Y ☐ N ☐ Did the pain occur at rest? Y ☐ N ☐
Pain the same as a previous AMI? Y ☐ N ☐ Is the patient still in pain? Y ☐ N ☐

PAST MEDICAL HISTORY

Myocardial infarction Y ☐ N ☐ How many? _____ Date of last MI: _____
Prior angina Y ☐ N ☐ Pacemaker/ICD Y ☐ N ☐
Previous PCI/stent Y ☐ N ☐ Date of last PCI: _____
Previous CABG Y ☐ N ☐ Date of last CABG: _____
Arrhythmia Y ☐ N ☐ Details (if known): _____

Risk factors:

Hypertension Y ☐ N ☐ Hyperlipidaemia Y ☐ N ☐ (Ever had or been treated for?)
Type 1 diabetes Y ☐ N ☐ Type 2 diabetes Y ☐ N ☐

Other past history

Stroke or TIA Y ☐ N ☐ Peripheral arterial disease Y ☐ N ☐ Heart failure Y ☐ N ☐
Renal impairment Y ☐ N ☐ Rheumatoid arthritis Y ☐ N ☐
Others: _____

MEDICATION HISTORY

Patient Name: _____ DOB _____ NUMBER _____

Aspirin ☐ Y ☐ N ☐ ACE inhibitor ☐ Y ☐ N ☐ Others: _____

Clopidogrel ☐ Y ☐ N ☐ Beta blocker ☐ Y ☐ N ☐

Prasugrel ☐ Y ☐ N ☐ Ca²⁺ blocker ☐ Y ☐ N ☐

Ticagrelor ☐ Y ☐ N ☐ Insulin ☐ Y ☐ N ☐

Oral nitrate ☐ Y ☐ N ☐ Nicorandil ☐ Y ☐ N ☐

Statin ☐ Y ☐ N ☐ Allergies: _____

RELEVANT SOCIAL HISTORY

Current smoking ☐ Y ☐ N ☐ Smoking in last 4/52 ☐ Y ☐ N ☐ Smoking ever? ☐ Y ☐ N ☐

Alcohol use? ☐ Y ☐ N ☐ More than 21u (men) or 14u (women)? ☐ Y ☐ N ☐

Family history of IHD? ☐ Y ☐ N ☐ (In a 1st degree relative aged <65 years)

ON EXAMINATION

Heart Rate: _____ Respiratory Rate _____ Blood pressure _____ SpO₂ _____ Temp _____

Heart sounds normal? ☐ Y ☐ N ☐ Details: _____

Respiratory examination:

Crepitations at bases? ☐ Y ☐ N ☐ Crepitations to mid-zones or above? ☐ Y ☐ N ☐

Killip Class: 1. No heart failure ☐ 2. Basal crackles or raised JVP ☐

3. Pulmonary oedema ☐ 4. Cardiogenic shock ☐

Other details: _____

Abdominal examination: Abdominal tenderness? ☐ Y ☐ N ☐

Other details: _____

Is the patient visibly sweating? ☐ Y ☐ N ☐

Is the chest wall tender? ☐ Y ☐ N ☐ Does palpation of the chest reproduce the pain? ☐ Y ☐ N ☐

Other: _____

IMPRESSION AND PLAN

Notes:

ECG interpretation:

Acute ischaemia ☐ Y ☐ N ☐ ST elevation (STEMI) ☐ Y ☐ N ☐ LBBB ☐ Y ☐ N ☐

ST depression ☐ Y ☐ N ☐ Abnormal T inversion ☐ Y ☐ N ☐ Dynamic changes? ☐ Y ☐ N ☐

How likely is ACS? (Tick the closest to your estimated probability)

Definite ☐ Probable ☐ Could be ☐ Probably not ☐ Definitely not ☐

Estimated probability of ACS (0-100%): _____

Disposition: After the ED, where is the patient going? (Or where did they go?)

Home ☐ OMU ☐ MAU/medical ward ☐ CCU/Cardiology ☐ Cath Lab ☐

TIME OF DECISION:

Treatment: In the ED, did the patient receive?..

Aspirin ☐ Clopidogrel ☐ Prasugrel ☐ Ticagrelor ☐ Fondaparinux/LMWH ☐

Thrombolysis ☐ Referral for primary PCI ☐ Morphine ☐ GTN ☐ Oxygen ☐

Chest X-ray: Requested? ☐ Y ☐ N ☐ Pulmonary oedema? ☐ Y ☐ N ☐ Upper lobe diversion? ☐ Y ☐ N ☐

Signature: _____ Print: _____

Appendix 2.4

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

THE BEST STUDY: 30-DAY FOLLOW UP

FOLLOW UP PROCEDURE:

All patients should be contacted by telephone, email, letter or visit after 30 days. If actual contact is made later than 30 days after their initial attendance, only the events occurring within 30 days of recruitment should be included in this follow up proforma.

'The initial visit' is defined as the initial attendance at the Emergency Department.

Step 1: Mortality status

Check health records to verify that the patient is still alive (e.g. NHS Spine, hospital and primary care records).

Has the patient died? Y ☐ N ☐

Date of death (if applicable): ____/____/____

Registered cause of death: _____

Step 2: Check the medical records

Check hospital records for details of all Emergency Department attendances, hospital admissions, outpatient appointments and relevant investigations.

If, on contacting the patient, they have attended another hospital, also obtain the medical records from that hospital.

Have all available electronic medical records been checked? Y ☐ N ☐

Now fill out Section 1 (below).

Step 3: Contact the patient

Unless the patient has died, contact the patient.

1. Method of contact with patient: Telephone ☐
 Email ☐
 Letter ☐
 Visit in person ☐

2. Date of contact (dd/mm/yyyy) ____/____/____

There are 3 parts to follow up: Clinical follow up; Health resource use; Quality of life.

Section 1: clinical follow up. First, we'd like to know if you've had any problems with your health over the last 30 days since you agreed to take part in this research. Over that time, have you had..? (Go through conditions listed in Section 2, below).

Section 2: resource use. We'd like to ask some questions about how much contact you've had with healthcare providers in the 30 days after you agreed to take part in the research on <date>. This will help us to work out the costs of your healthcare to the NHS.

Section 3: health status. Follow the EQ-5D telephone script guidance.

Now complete the relevant sections (below).

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

Section 1: Clinical follow up

2. Date of discharge from hospital (dd/mm/yyyy): ____/____/____

		Total number of nights spent in an Emergency Department observation ward	Number of nights spent in a general medical ward	Number of nights spent in an Intensive Care Unit	Number of nights spent in a Coronary Care Unit
During the initial hospital stay:	Details from medical records				
	Details from direct patient contact				

3. Has the patient had:

a. An acute myocardial infarction? Y ☐ N ☐ Date: ____/____/____

b. A cardiac arrest? Y ☐ N ☐ Date: ____/____/____

c. An angiogram demonstrating $\geq 50\%$ stenosis that is not known to be old? Y ☐ N ☐ Date: ____/____/____

d. Angioplasty or PCI? Y ☐ N ☐ Date: ____/____/____

Did this take place during the initial admission? Y ☐ N ☐

e. Cardiac bypass surgery? Y ☐ N ☐ Date: ____/____/____

Did this take place during the initial admission? Y ☐ N ☐

f. A stroke? Y ☐ N ☐ Date: ____/____/____

g. An arrhythmia? Y ☐ N ☐ Date: ____/____/____

Details: _____

h. Acute decompensated heart failure? Y ☐ N ☐ Date: ____/____/____

i. Any tests for heart disease? Y ☐ N ☐ Date: ____/____/____

Investigation (Obtain all reports)	Done as in-patient (date)	Done as out-patient (date)	Not done
Echocardiography			
ETT			
Myoview (thallium scan)			
Stress echo			
Angiogram			
Other (state)			

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

j. Bleeding? Y ☐ N ☐ Date: _____

Details: _____

Drop in Hb > 2g/dL Y ☐ N ☐ Date: _____

Blood transfusion for bleeding Y ☐ N ☐ Date: _____

Lowest recorded haemoglobin _____

k. Renal failure needing dialysis? Y ☐ N ☐ Date: _____

l. Other event? Y ☐ N ☐ Date: _____

Details: _____

Source(s) of the above data: Medical records ☐ Direct patient contact ☐

2. Has the patient had:

a. Any adverse event? Y ☐ N ☐

b. Any serious adverse event? Y ☐ N ☐ (Fill out AE/SAE)

Details: _____

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

Section 2: Healthcare resource use

1. "Since your initial visit, have you had **any** further contact with health services?" (Including any visits to hospital, any contact with their GP, nurse or social centre, and any visits to Urgent Care Centres or Walk in Centres?)

YES ☐ NO ☐

If YES, proceed to question 2. IF NO, proceed to EQ-5D (page 7)

2. "Since your initial visit, how many times have you attended an Emergency Department (A&E)?"

_____ times

2b. How many of these attendances were for chest pain? _____ times

2c. On how many occasions were you admitted to hospital? _____ times

Source of this data: Medical records ☐ Direct patient contact ☐

3. "Since your initial visit, have you stayed overnight in hospital?"

(Including those hospital admissions counted in 2c)

YES ☐ NO ☐

For each admission, please record the number of nights the patient stayed in the hospital and the number of nights they stayed in an Intensive Care Unit or a Coronary Care Unit. Wherever possible, verify all patient reports against the **medical records**. Record information obtained from the **medical records** and from **direct contact with the patient** separately, so that we know how the data were obtained.

	Total number of nights spent in an Emergency Department observation ward	Number of nights spent in a general medical ward	Number of nights spent in an Intensive Care Unit	Number of nights spent in a Coronary Care Unit
Admission 1				
Admission 2				
Admission 3				
Admission 4				
Admission 5				

Source(s) of this data: Medical records ☐ Direct patient contact ☐

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

4. "Since your initial visit, have you patient *visited* any other healthcare providers?"

YES ☐ NO ☐

If so, how many times has the patient visited each of the following?

Provider	Number of visits	Main reason for visit
General practitioner		
Practice nurse (on GP premises)		
Urgent Care Centre		
Walk In Centre		
Hospital out patient appointment		
Other (1) _____		
Other (2) _____		
Other (3) _____		

Source(s) of this data: Medical records ☐ Direct patient contact ☐

5. "Since your initial visit, have you *had a home visit from* any other healthcare providers?"

YES ☐ NO ☐

If so, how many times has the patient *been visited* by each of the following?

Provider	Number of visits	Main reason for visit
General practitioner		
Practice nurse (on GP premises)		
Urgent Care Centre		
Walk In Centre		
Hospital out patient appointment		
Other (1) _____		
Other (2) _____		
Other (3) _____		

Source(s) of this data: Medical records ☐ Direct patient contact ☐

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

6. "Since your initial visit, have you *had telephone contact with any other healthcare providers?*"

YES ☐ NO ☐

If so, how many times has the patient *had telephone contact with* each of the following?

Provider	Number of visits	Main reason for visit
General practitioner		
Practice nurse (on GP premises)		
Urgent Care Centre		
Walk In Centre		
Hospital out patient appointment		
Other (1) _____		
Other (2) _____		
Other (3) _____		

Source(s) of this data: Medical records ☐ Direct patient contact ☐

Any further information about contact with health services:

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

8. Is the patient taking any new medications since hospital discharge?

Medication	Dose and route (if known)	Frequency	Approximate start date

Source(s) of this data: Medical records ☐ Direct patient contact ☐

Any other information:

Signed: _____ Print: _____ Date: _____

PATIENT STUDY NUMBER: _____ FOLLOW UP DATE: _____

Section 3: Health status

Enter what the patient describes best matches the participant's health TODAY.
(Refer to EQ-5D telephone script)

1. Mobility

- a. I have no problems walking about ☐
- b. I have some problems walking about ☐
- c. I am confined to bed ☐

2. Self-care

- a. I have no problems with self-care ☐
- b. I have some problems washing or dressing myself ☐
- c. I am confined to bed ☐

3. Usual Activities

- a. I have no problem performing my usual activities ☐
- b. I have some problems performing my usual activities ☐
- c. I am unable to perform my usual activities ☐

4. Pain or discomfort

- a. I have no pain or discomfort ☐
- b. I have moderate pain or discomfort ☐
- c. I have extreme pain and discomfort ☐

5. Anxiety or depression

- a. I am not anxious or depressed ☐
- b. I am moderately anxious or depressed ☐
- c. I am extremely anxious or depressed ☐

From 0 (worst possible) to 10 (best possible), how would you best describe your health state today? /10

Signed: _____ Print: _____ Date: _____

Appendix 2.5

The BEST (Bedside Evaluation of Sensitive Troponins) Study

Central Manchester University Hospitals 
NHS Foundation Trust

You are being invited to take part in a research study. You may have already told us that you are happy to take part. We do this so that your treatment is not delayed. To include you in the study, we also need your permission in writing. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read this information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

1. WHAT IS THE PURPOSE OF THE STUDY?

When people suffer from chest pain, we are often worried that the pains may be coming from a heart problem such as a heart attack. It is often difficult to tell quickly if a patient has pain from a heart problem or from something less serious like a muscle strain. At the moment, we usually do at least two blood tests to look for signs of heart damage. The blood samples are tested in the main laboratory and it can take up to 2 hours for the results of each test to be available.

It is now possible to do the blood tests for signs of heart damage using machines that can be kept in the Emergency Department (A&E). We call these machines 'point of care devices'. When we use them, we can usually get a blood test result just 20 minutes after taking blood.

In this research study, we want to find out whether the results that we get from the new machines are as accurate as the results that we get from the main laboratory. We also want to carefully analyse the results to work out the quickest, safest and best ways to identify patients who are having a heart attack. We hope that this research will enable us to provide faster and better care to patients in the future.

2. WHY HAVE I BEEN CHOSEN?

Your doctor is concerned that your chest pain may be caused by heart disease and wants you to have further tests. The new tests are designed to be used in exactly this situation.

3. DO I HAVE TO TAKE PART?

No, you can decide whether or not to take part. If you do decide to take part, we will give you this information sheet to keep and we will ask you to sign a consent form. You can still withdraw at any time without giving a reason. If you decide not to take part, or if you decide to withdraw from the study, it will not affect the standard of care you receive.

4. WHAT WILL HAPPEN TO ME IF I TAKE PART?

If you take part in this study you will still receive exactly the same treatment. Your treatment will not be delayed or changed if you decide to take part. As part of this study we will take

Patient information sheet version 3.1. November 16, 2015. Page 1 of 3
Full Study Title: The bedside evaluation of sensitive troponins (BEST) study

research team can access. We will not record details that could be used to identify you (such as your name or date of birth) on blood samples, in our main database or when we publish the results of this research. In order to monitor the conduct of the study, responsible experts from the sponsor or regulatory authorities may need access to your original records under strict observance of confidentiality.

With your permission, we will let your general practitioner know about your involvement in the study. This is because (a) we may need to contact your GP for follow up information if it is not possible to get in touch with you directly and (b) from time to time, your doctor may know of a reason why it may not be in your best interests to be involved in our research, and they can let us know about this.

9. WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH?

The results of this research are likely to be published by 2017 in a medical journal, which will enable more people to benefit from the findings. All participants in the study will remain anonymous. If you would like to hear about the results, please let us know and we will write to you when they are available.

10. WHO IS ORGANISING AND FUNDING THE RESEARCH?

This research is receiving support from the National Institute for Health Research, Manchester Metropolitan University and Horizons-2020 (a research grant from the European Union that we obtained in collaboration with a company called FABPulous, which manufactures point of care tests). Manchester Metropolitan University has received funding from the Saudi Arabian government to support a PhD student for this research. We also expect to receive some funding from Abbott Point of Care, a company that manufactures point of care devices. We will not share any information that could be used to identify you with the funders of this research. The research is sponsored by Central Manchester University Hospitals NHS Foundation Trust.

11. WHO HAS REVIEWED THE STUDY?

The study has been reviewed and approved by the North West Greater Manchester Central Research Ethics Committee, reference 14/NW/1344

12. CONTACT FOR FURTHER INFORMATION

For further information about the study, you may wish to contact:

Dr Richard Body, Emergency Department, Manchester Royal Infirmary, Manchester, M13 9WL

Email richard.body@cmft.nhs.uk; Tel: 0161 276 4147; Secretary 0161 276 8539

Alternatively, you may contact the Patient Advice and Liaison Service as an independent contact point:

Patient Advice and Liaison Service (PALS) Co-Ordinator, Manchester Royal Infirmary, Manchester, M13 9WL.

Email pals@cmft.nhs.uk. Telephone 0161 276 8686.

You will be given a copy of the information sheet and a signed consent form to keep.